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Further Effects of Androgenic Substance on Sexual Development in the Female White Rat.

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The production of intersexed rats by administration of testosterone to the pregnant mother of these animals has been reported.[1] One hundred and fifty-two female albino rats have been injected with varying amounts of testosterone and testosterone propionate* at different periods of pregnancy. The testosterone has been given by multiple subcutaneous injections, the testosterone propionate by both single and multiple injections. The pregnancy of 97 of these rats has terminated by resorption or by delivery of necrotic fetuses. Fifty-five have delivered at term, spontaneously or by cesarean section. Females of 19 litters have survived to maturity and have been autopsied. In addition, females of 10 litters whose mothers have received high dosages are now living. Of the 19 litters autopsied, the females of 7 litters were normal. The mothers of these animals received apparently subthreshold dosages (1.65 to 3.5 mg. of plain testosterone and 0.5 to 2.5 mg. of testosterone propionate).

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[1] Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200.

* We wish to thank Dr. Schwenk of Schering Corporation for the generous supply of testosterone and testosterone propionate used in this work.

It was stated in our previous report that well affected adult animals had no distal vagina and that the blindly ending upper vagina and the uteri may become distended with retained secretions. This has been confirmed by Hamilton and Gardner.[2] In 8 adult intersexed animals since obtained whose mothers have received higher dosages (5.0-9.0 mg. testosterone propionate and 13.5 mg. plain testosterone), the uteri and upper vagina were not distended. Serial sections and wax reconstructions of 2 of these animals have revealed that the vagina communicates with the urethra by bilateral ostia medial to the ostia of the ejaculatory ducts. Identical conditions have been found in serial sections of 3 new-born whose mothers received similar dosages. This condition may be logically interpreted as a preservation of the original bilateral junctions of the mullerian ducts with the urogenital sinus. Serial sections of a new-born whose mother received smaller dosages (3.5 mg. testosterone propionate) reveal bilateral communication of the epithelium of the vagina with the urethra, but with this dosage the communication is represented by a non-patent solid cord of cells. The adult litter mate of this animal displayed the typical utero-vaginal distention associated with this condition.

In our previous communication it was stated that inhibition of mullerian duct derivatives had been obtained. This evidence has been limited, however, to 2 serially sectioned new-born. We have been unable to confirm these findings, since in 46 new-born and 21 adult intersexed animals examined to date, uteri and oviducts are present.

The presence in adult intersexes of structures that resembled seminal vesicles was reported. Such structures have been identified in 8 adult animals. A further development of wolffian duct derivatives has been subsequently noted. Examination under a dissecting microscope of new-born animals whose mothers have received high dosages (5.0-14.0 mg. testosterone propionate and 13.5 mg. plain testosterone) has revealed a persistence of the vas deferens adjacent and parallel to the uterus. This structure was present on one side in 10 animals and on both sides in 15 animals. The wolffian duct derivatives are present from gonad to urethra as was demonstrated by serial sections, graphic and wax plate reconstructions. Efferent tubules connect with the rete of the gonad and continue into a well marked epididymis, vas deferens, and ejaculatory duct. Anlage of the seminal vesicles are present, but seem to be displaced cephalad so that the ejaculatory duct is relatively longer than the ejaculatory duct of a normal new-born male. In 3 experimental animals (mother given 6.0 mg. testosterone propionate) at an adult age, efferent tubules, head and tail of the epididymis, vas deferens, seminal vesicle, various prostatic lobes, Cowpers glands and a penis were present combined with apparently normal oviducts, uteri and upper vagina.

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[2] **Hamilton, J. B., and Gardner, W. A., PROC. SOC. EXP. BIOL. AND MED., 1937, 37, 570.**

Study of the peni of these animals is as yet incomplete but an *os priapi* which is found in the normal adult male is present.

The gonads of adult animals whose mothers received low dosage of the hormone are grossly normal with visible corpora lutea. The gonads of animals prenatally affected by higher dosages are smaller and present a smooth white surface. Corpora lutea are not grossly visible and microscopic examination shows that they are absent. Follicles are present. An extensive rete is present and medullary tubules extending from the rete into the stroma of the gonad have been noted. A more detailed histological study of the gonads is in progress.

Summary. Sixty-seven intersexed rats, produced by injecting the pregnant rat with testosterone, have been examined. Testosterone given to the pregnant rat may in some instances arrest the fusion of the caudal portions of the mullerian ducts and the unfused portions are patent and open into the urethra. In addition, a number of animals have been produced in which wolffian duct derivatives, efferent tubules, epididymis, vas deferens and seminal vesicles are present in association with a fallopian tube, uterus and the upper portion of the vagina and with masculinization of the female gonad.

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Masculinization of Female Rats by Postnatal Administration of Male Sex Hormone.

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The production of intersexuality in rats has been reported.[1, 2] These animals were obtained by injecting the pregnant mother with male sex hormone during pregnancy. Only the genetically female offspring were affected. Well affected animals had a penis instead of a clitoris and no external or lower vagina. These animals also had prostates and seminal vesicles. Dantchakoff has also obtained similar changes in the embryonic guinea pig by injection of testosterone propionate directly into the amniotic cavity. Growth and modification of the clitoris in castrated females into which testes have been transplanted has been reported.[3] During the progress of this work Lacassagne and Raynaud[4] have reported the production of a penis-like organ in female mice injected continuously from birth to 6 weeks with testosterone propionate.

The embryological development of rats is relatively slow and the offspring are born in an almost fetal state. In the female new-born for example, the lower vagina is represented by a solid cord of epithelium that caudally merges with the posterior epithelium of the urethra which empties at the primary urogenital ostium. The clitorine urethra (which is present in the normal adult female rat) is not completely formed until 48 to 72 hours postpartum.

It seemed possible that developmental changes could be produced by injecting the female new-born with male sex hormone. Accordingly new-born female rats were injected with small doses of testosterone (0.1 to 0.3 mg. per rat in a single dose) within 24 hours after birth. No changes were obtained in these animals. Another series of animals were then injected with very large doses (2.5 to 17.5 mg. per rat, total dosage) of testosterone propionate* at 2- or 3-day intervals until the twenty-first day after birth. Nor-

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[1] Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 2226.

[2] Greene, R. R., Burrill, M. W., and Ivy, A. C., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 1.

[3] Lipschutz, A., *Arch. J. Entw. Mechan.*, 1918, **44**, 196.

[4] Lacassagne, A., and Raynaud, A., *Compt. Rend. Soc. de Biol.*, 1937, **125**, 351.

* We wish to thank Dr. Schwenk of Schering Corporation for the generous supply of testosterone and testosterone propionate used in this work.

mal control littermates and the treated animals were killed and autopsied after maturity. The findings are shown in Table I.

TABLE I.

Litter No.	No. Treated Females	No. Control Females	Total Dosage Given	Days Dosage Given	Phallus	Vagina
12	2	3	0.1T	1st	Normal	Normal
	1		0.2T	1st	,,	,,
13	1	1	0.3T	1st	,,	,,
14	3	3	0.3T	1st	,,	,,
17	1	2	0.3T	1st	,,	,,
53	2	2	2.5TP	21	Penis-like	Minute orifice base of phallus
40	3	2	10.5TP	21	,, ,,	Urethra and vagina had common opening at base of glans
37	2	2	12.0TP	21	,, ,,	No vaginal orifice
36	3	3	12.0TP	21	,, ,,	,, ,, ,,
30	3	0	17.5TP	21	,, ,,	,, ,, ,,

The control animals were normal in all respects. The injected animals receiving the large doses had masculinized external genitalia, inasmuch as the phallus resembled a penis and the development of the external vagina was inhibited. It is to be noted that with the highest dosages (12-17.5 mg. of testosterone propionate) the vagina ended blindly under the perineal skin. With a dosage of 10.5 mg. the vagina and urethra had a common opening at the base of the phallus. These openings were not visible externally, but were covered by apparently normal preputial skin. With 2.5 mg. of testosterone propionate the vaginal orifice was present as a minute opening through the preputial skin at the caudal base of the phallus. The phallus in these affected animals resembled a normal penis insofar as the development of the corpora cavernosa was concerned, but the corpus spongiosum urethræ was not as well developed and the phallus was bent caudally. An *os priapi,* which is present in the normal male rat, was found in the affected animals. A right ventral lobe of the prostate (para-urethral gland) was observed under a dissecting microscope in 3 of the affected animals.

Conclusion. The administration of a sufficient quantity of testosterone propionate to the new-born female rat will cause a masculinization of the external genitalia.

9718 P

Carbohydrate Utilization in the Hypophysectomized Dog.*

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We have recently demonstrated the existence of a direct relationship between the height of the blood sugar level and the rate of sugar utilization in normal dogs. We have also shown that this same relationship persists (with some quantitative differences) during the absence of insulin, after total pancreatectomy in dogs.[1] These facts render it imperative to consider the characteristic blood sugar levels of different physiological preparations in any attempt to compare their relative abilities to utilize carbohydrate. The present preliminary report concerns the influence of the removal of the hypophysis upon sugar utilization, in the light of the above considerations.

The utilization of carbohydrate at various blood sugar levels in eviscerated hypophysectomized dogs, was determined under the same nutritional and experimental conditions and using the same methods of calculation as detailed in our previous publication.[1] The animals had been hypophysectomized 4 to 8 weeks previously by a method slightly modified from that of Dandy.[2] The functional lack of the gland was indicated by the hypoglycemic effects of fasting[3] before the experiments, and its anatomical absence was confirmed by autopsy examinations after the experiments.

The results are plotted in Fig. 1, where they may be compared to the smooth curves representing our previous results in normal and depancreatized animals. It may be seen that the hypophysectomized dogs utilized less sugar than the normal animals, at any given blood sugar level. They also used less sugar than the depancreatized animals at the blood sugar levels ordinarily maintained by the diabetic organism (*i. e.*, above 350 mg. %). More specifically, the hypophysectomized dog at its usual post-absorptive blood sugar levels (35-55 mg. %) utilizes carbohydrate about one-half as effectively as the normal dog in the normal blood sugar range (60-

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* Aided by the Max Pam Fund for Metabolic Research.

[1] Soskin, S., and Levine, R., *Am. J. Physiol.*, 1937, **120**, 761.

[2] Dandy, W. F., and Reichert, F. L., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 1.

[3] Soskin, S., Mirsky, I. A., Zimmerman, L. M., and Crohn, N., *Am. J. Physiol.*, 1935, **114**, 110.

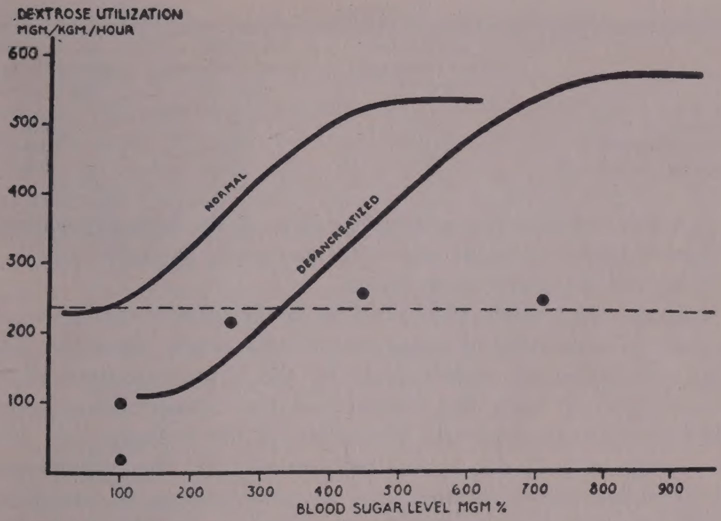

FIG. 1.

Each black dot represents an experimental result on a different hypophysectomized dog. The smooth curves, included for comparison, are derived from a larger number of similar experiments previously reported.[1] The broken horizontal line indicates the rate of dextrose utilization of normal dogs within the normal glycemic range.

80 mg. %). Within the range of blood sugar levels usually maintained by well nourished "Houssay" animals (350-550 mg. %)[4] the hypophysectomized animal utilizes sugar at rates which vary from that of the depancreatized animal to two-thirds of that rate.

Some of our data also indicate the modified intermediary processes which accompany the diminished use of carbohydrate after hypophysectomy. Thus, a comparison of the average rate of fall of muscle glycogen values, for all the experiments in our previous[1] and present work is as shown in Table I.

TABLE I.

	Av. Initial Muscle glycogen mg./%	Av.Final Muscle glycogen mg./%	Av. Decrease in Muscle glycogen mg./%/hour
Normal	511	355	43.1
Depancreatized	337	217	38.4
Hypophysectomized	584	570	4.1

A similar comparison of the average rate of rise in blood lactic acid levels is as shown in Table II.

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[4] Soskin, S., Mirsky, I. A., Zimmerman, L. M., and Heller, R. E., *Am. J. Physiol.*, 1936, **114**, 648.

TABLE II.

	Av. Initial Blood lactic acid mg./%	Av. Final Blood lactic acid mg./%	Av. Increase in Blood lactic acid mg./%/hour
Normal	50.5	106.7	15.2
Depancreatized	118.1	183.2	21.0
Hypophysectomized	27.3	62.8	9.5

It is evident that the muscle glycogen of the hypophysectomized animal is relatively stable and is not as rapidly catabolized as in the normal and depancreatized animal.

Taking into account the influence of the blood sugar level upon the rate of utilization of sugar, our results clearly show the diminished utilization of carbohydrate by the hypophysectomized dog, as compared to both the normal and the depancreatized animal. These results are important in explaining the hypoglycemic effects of fasting in both the hypophysectomized and the hypophysectomized-depancreatized animal, and the amelioration of the diabetic syndrome in the latter animal. It is obvious that these effects of hypophysectomy cannot be due to an increased rate of sugar utilization. Sugar utilization is actually diminished and, therefore, the hypoglycemia and modification of the diabetes must be attributed to a proportionately greater decrease in the rate of new formation of sugar by the liver. This work confirms and extends our previous report upon the influence of hypophysectomy on gluconeogenesis in the normal and depancreatized dog.[3]

9719 P

A Method for Isolation of Ascorbic Acid Fermenting Bacteria.

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The objectives of this preliminary communication are two: to describe a procedure for the isolation of ascorbic acid fermenting bacteria, from suitable material, and to describe briefly the results obtained with this procedure. This preliminary communication does not presume completeness either with respect to the types or strains of bacteria which may actually utilize ascorbic acid for energy, or with regard to the patterns of fermentation of this substance by specific strains of bacteria.

Plain, nutrient broth is reinforced with ascorbic acid.* Fifteen to 20 mg. per 10 cc. of broth is a preferable amount, both because this is sufficient to indicate unequivocally that decomposition has taken place, and also because within these limits the initial reaction of the broth is not brought below pH 6.0, a point at which the bacteria thus far encountered grow readily. Also, a reaction slightly on the acid side is conducive to the stability of ascorbic acid. The broth, thus prepared is inoculated with stomach contents, feces, or other suitable material and incubated at 37° for 18-30 hours. Then, if ascorbic fermenting bacteria are present, a considerable growth will be noticed. From this first, or preliminary culture, a second broth culture is made, using 2 to 5 loops for the inoculum. After incubation and growth, plates are made either upon plain, or, preferably, glucose agar. (Ascorbic acid agar is not so well suited for this purpose.) Colonies are picked off, tested for purity and eventually for ascorbic acid utilization. The use of 2 successive cultivations in ascorbic acid broth is desirable both because it tends to eliminate non-fermenting organisms, and because it acclimatizes the bacteria to the medium. Inasmuch as bacteria isolated by this procedure are somewhat prone to dissociation some stability is obtained. Using this method, some 30 cultures of bacteria have been isolated, without difficulty, from gastric contents of achlorhydrics (a fruitful source of ascorbic acid fermenting bacteria) from a normal artificially fed baby, from the feces of a normal adult, and from the feces of guinea pigs. The cultures obtained are of 2 types, with some individual variation in minor details: members of the *Lactis aerogenes,* or pneumobacillus group, and members of the enterococcus group. These have been tested and retested for their ability to decompose ascorbic acid as follows:

Ninety cc. of plain broth, in an Erlenmeyer flask, is inoculated with the desired culture and incubated 18 hours, at which time a good growth is obtained. Ten cubic centimeters of ascorbic acid solution, filtered through a Berkefeld filter to insure sterility, and of such strength that the final concentration in the broth will be from 15 to 20 mg. per 10 cc. broth is added, and the medium is distributed in a series of sterile test tubes (preferably ⅝ inches in diameter or less, to reduce chances of oxidation), using exactly 10 cc. to each tube. These several tubes, together with control tubes containing exactly the same proportion of ascorbic acid, but no bacteria, are incubated, and at appropriate intervals examined for their respective amounts of this substance. The method of quantitation of ascorbic acid is as follows: At the proper time, exactly 1 cc. of culture is removed, and mixed with approximately 1 cc. of glacial acetic acid to arrest growth. An equal amount of control uninoculated broth is treated in exactly the same manner. Each solution is then made up to precisely 50 cc. with distilled water. The most direct way is to place the culture (1 cc.) in a 50 cc. volumetric flask, add the acetic acid, then make up to the 50 cc. mark with water. Two aliquots, of 1 cc. each, are taken for analysis by titration against a freshly prepared, standardized solution of 2:6 dichlorophenolindophenol dye of which each cubic centimeter is equivalent to 0.02 mg. ascorbic acid. The end point for the titration is the first appearance of a faint, but distinct bluish color. From the amount of ascorbic acid medium required to elicit this color, it is a very simple matter to calculate the amount of ascorbic broth in the solution. It is good practice to repeat the determination of ascorbic acid upon a sample which has been saturated with H_2S prior to titration, thereby changing any reversibly oxidized ascorbic acid to the fully reduced, and titratable condition, and thus acting as an additional check on the decomposition of the substance by the bacteria.

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* The ascorbic acid was very generously provided by Merck and Co., Inc.

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Pathogenesis of Arterial Hypertension in Coarctation of the Aorta.

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Experiments on albino rats show that the hypertension in coarctation of the aorta has the same pathogenesis as the hypertension in a Goldblatt dog (partial constriction of a renal artery). The criterion of hypertension was the presence of cardiac hypertrophy estimated in relation to body weight (left ventricle more than right) in non-anemic rats sacrificed 20 days after operation. Blood urea concentrations were normal and proteinuria was not increased over the control levels.

The experimental procedure consisted in a modification of Collins'[1] technique. The left renal artery was tied together with a wire 0.4 mm. diameter, or the aorta with a wire 0.9 mm. diameter; after tying, the wire was removed but the ligature left in place.

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[1] Collins, D. A., *Am. J. Physiol.*, 1936, **116**, 616.

Such partial occlusion of the left renal artery was followed by cardiac hypertrophy (average +28%) in 9 rats. When there was simultaneous left nephrectomy there was of course no hypertrophy (+2%) in 14 rats.

Partial occlusion of the aorta between the kidneys resulted in cardiac hypertrophy in 7 rats (+26%); but when left (distal) nephrectomy was done simultaneously, cardiac hypertrophy failed to occur (−2%) in any of 25 rats. In some cases the occlusion had become complete, assurance that the ligature was sufficiently tight.

Finally, partial occlusion of the aorta above both kidneys resulted in cardiac hypertrophy (+20%) in 12 rats. This situation is analogous to that existing in coarctation of the aorta.

Conclusions. Partial (or even complete) occlusion of the aorta in rats produces hypertension only if there is living renal tissue distal to the occlusion, just as there must be a kidney beyond a partially occluded renal artery in order to produce hypertension in a Goldblatt dog. The same degree of mechanical obstruction due to stenosis of the aorta and the presence of a collateral bed never results in hypertension when all of the renal tissue is above the site of occlusion.

9721

Study of Some Variables Affecting the Prothrombin Time.*

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Of the 4 substances in the plasma (calcium, fibrinogen, thromboplastin and prothrombin) only 2, calcium and fibrinogen, can be estimated quantitatively. Since thromboplastin is derived presumably from platelets, estimations of the number and fragility of platelets are considered to be adequate tests for determining the presence of sufficient thromboplastic substance. Although prothrombin can be isolated from the blood in relatively pure form, the methods for its isolation are not adaptable to a study of fluctuations under pathologic and experimental conditions. Therefore, in determining the amount of prothrombin in a sample, it has been necessary to use methods of bioassay which depend upon a comparison of coagulation times. Howell[1] uses the coagulation time of recalcified plasma as the index of the amount of prothrombin present. This method may be criticized because it records variations not only in prothrombin but also in thromboplastin. The method of Quick, *et al.*,[2] by addition of an excess of thromboplastic substance, presumably leaves the prothrombin concentration as the only variable in the reaction.

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* Assisted by a grant from the Christine Breon Fund.

However, other variables must be considered. Warner, *et al.*,[3] recognized the variations in the Quick reaction caused by substances other than prothrombin. In their prothrombin test, a globulin fraction of the plasma, presumably containing almost all of the prothrombin, is precipitated from the plasma, redissolved in oxalated saline, dialyzed, and then tested in a constant mixture of calcium-thromboplastin-fibrinogen. This method probably eliminates substances other than prothrombin which might influence the reaction. It should be noted, however, that substances in combination with plasma proteins that are incapable of removal by dialysis may be precipitated in the globulin fraction and may still influence the reaction. Our experiments show that the addition of various substances alters the prothrombin time as measured by the method of Quick.

The method of Quick,[2] with minor variations, was employed. Nine cc. of venous blood were mixed with 1 cc. of M/10 sodium oxalate, and centrifugalized; and the supernatant plasma was removed. One-tenth cc. of this plasma was mixed with 0.1 cc. of M/40 $CaCl_2$ and 0.1 cc. of fresh rabbit brain emulsion in a 13 mm. tube at 37°C.; and the time of coagulation was recorded with a stopwatch. In the control tests, 0.1 cc. of 0.85% NaCl was substituted for the brain emulsion. The emulsified brain was centrifugalized. This undiluted material represented a 100% emulsion, and from it serial dilutions to 0.001% were made. Progressively increasing concentrations were used to demonstrate any effect of an excess of thromboplastic material. Using the supernatant saline solution from similar emulsions up to a 20% concentration, Quick[4] demonstrated that the coagulation time could not be shortened beyond a minimum which was reached with a 10% emulsion. Each of Tables I to VII presents the results obtained in one of a series of experiments. In each table the figures shown were characteristic of all experiments of the series.

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[1] Howell, W. H., *Arch. Int. Med.*, 1914, **13**, 76.

[2] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J Med. Sci.*, 1935, **190**, 501.

[3] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667

[4] Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282.

Table I demonstrates the apparent anticoagulant action of high concentrations of thromboplastic material.

TABLE I.
Effect of Progressively Increasing Concentrations of Rabbit Brain Emulsion on Coagulation Time of Normal Recalcified Plasma.
(Results in one of 15 experiments.)

% Brain Emulsion	100	75	50	25	20	15	10	5	4	3	2	1	½	.1	.01	.005	.001	0.85% NaCl Control
Coag. Time in Seconds	40	30	*25*	*23*	*23*	*24*	*23*	*24*	*23*	*23*	*22*	*25*	*26*	40	63	73	74	120

Table II shows that the inhibition of coagulation in high concentrate is not due to an excess of thromboplastin but is rather a physical characteristic of the reaction.

TABLE II.
Effect of Progressively Increasing Concentrations of Rabbit Brain Emulsion on Coagulation Time of Diluted* Normal Recalcified Plasma.
(Results in one of 5 experiments.)

% Brain Emulsion	100	75	50	25	20	15	10	5	4	3	2	1	½	.1	.01	.005	.001	0.85% NaCl Control
Coag. Time in Seconds	900	240	90	65	55	48	44	*38*	*37*	*37*	*38*	*42*	45	68	85	97	124	138

* A mixture of 40% plasma and 60% of a solution of 0.85% NaCl containing the same concentration of sodium oxalate as the original plasma.

If the prolonged coagulation time in high concentrations of brain emulsion were due to an excess of thromboplastin, there would be a gradual shift of the optimum concentration of brain emulsion toward the lower concentrations with progressive dilution of the plasma. It is apparent this is not true. The optimum concentration of rabbit brain emulsion was found to be between 0.5% and 50% for normal plasma. This optimum range was markedly narrowed by the use of any plasma exhibiting a prolonged coagulation time. Table II demonstrates narrowing of the optimum range with dilution of the plasma, and Table III narrowing of the range in icteric plasma.

A 5% rabbit brain emulsion supplied the optimum concentration of thromboplastic material in all conditions studied. In no instance was the rabbit brain perfused. While this omission may cause a slight prolongation of the coagulation time, it allows comparison with thromboplastic material from sources in which perfusion is not possible.

TABLE III.
Effect of Progressively Increasing Concentrations of Rabbit Brain Emulsion on Coagulation Time of Icteric Recalcified Plasma.
(Results in one of 15 experiments.*)

% Brain Emulsion	100	75	50	25	20	15	10	5	4	3	2	1	½	.1	.01	.005	.001	0.85% NaCl Control
Coag. Time in Seconds	600	150	120	55	51	44	*39*	*35*	*36*	*35*	*38*	*40*	45	54	78	104	125	180

*The coagulation time was similarly prolonged in all cases studied. The degree of prolongation varied with each individual case.

In all experiments, the oxalated blood was centrifugalized at 1800 r.p.m. for 10 minutes, and the supernatant plasma removed. There was no significant change in coagulation time whether high or low speed centrifugalization was used.

To test the effect of added substances on the coagulation time, the following method was employed: Equal quantities of a M/20 $CaCl_2$ solution and a solution of the substance to be tested, made up in twice the final concentration desired, are mixed. This produces a solution in which the $CaCl_2$ concentration is M/40, and the substance to be tested is of the desired concentration. Since in the performance of the test the $CaCl_2$ solution and the plasma are present in equal volume, any effect noted after the addition to the $CaCl_2$ solution of a foreign substance may be interpreted as if the substance were added directly to the plasma at the time of recalcification.

The coagulation time of normal plasma by this method is 22 to 25 seconds (Table I); that of icteric or diluted plasma may be prolonged to 70 seconds. Table II shows prolongation of the coagulation time in normal plasma diluted to 40%, and Table III in a case of obstructive jaundice (icterus index 110).

Table IV demonstrates progressive prolongation of the coagulation time after the addition of increasing amounts of heparin.

TABLE IV.
Effects of Progressively Increasing Concentrations of Heparin* on Coagulation Time of Normal Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion.
(Results in one of 4 experiments.)

Units of Heparin per cc. plasma	Normal control	0.1	0.2	0.3	0.5	1	2	3	4
Coagulation time in seconds	22	22	23	26	29	37	52	110	140

*Connaught Laboratories—1000 units per cc.

As is shown in Table V, increasing quantities of NaCl caused a progressive prolongation of the coagulation time even in the presence of an optimum concentration of all of the elements necessary for coagulation of the plasma.

TABLE V.
Effect of Progressively Increasing Concentrations of NaCl on Coagulation Time of Normal Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion.
(Results in one of 4 experiments.)

NaCl added	Control	.10N	.20N	.30N	.40N	.50N	.60N	.70N	.80N	.90N
Coagulation time in seconds	22	24	27	30	33	37	42	52	65	72

Quick,[5] using a thrombin-fibrinogen mixture, demonstrated that coagulation is definitely altered by the presence of electrolytes. That there is a qualitative difference between various simple salts, independent of the total concentration of electrolytes, is demonstrated in Table VI.

TABLE VI.
Effect of Increasing Progressively the Concentrations of $CaCl_2$ on the Coagulation Time of Normal and Icteric Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion, the Total Electrocyte Content Being Held Constant.*
(Results in one of 4 experiments.)

% CaCl	.05	.10	.15	.20	.25	.30	.35	.40	.50	.60	.70	.80	.90	1.00
Icteric Plasma Time in Seconds	74	*44*	*41*	*41*	*42*	*42*	48	53	62	70	84	87	93	122
Normal Plasma Time in Seconds	65	*30*	*32*	*30*	*32*	*35*	37	39	42	47	55	60	65	67

*Solutions made by mixing 0.35N $CaCl_2$ (1.925%) and 0.35N NaCl (1.225%) in varying quantities to attain the desired calcium concentration.

In this instance the concentration of Cl ion remains constant while the quantities of Na and Ca ion are reciprocally varied. Even though the total electrolytes are held constant, there is a progressive lengthening of the coagulation time with increasing quantities of Ca ion. The optimum concentration of Ca for recalcification of oxalated plasma is the same in normal as it is in icteric blood.

The effect of the addition of complex salts to the plasma is more striking (Table VII).

The addition of 400 mg. % ox bile salts (sodium taurocholate and sodium glycocholate) to normal plasma produces the same alteration in coagulation time as is found in icteric plasma. While this concentration of bile salts is above that usually stated to be present in the blood of patients with obstructive jaundice (6 to 20 mg. %), it must be remembered that no accurate quantitative

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[5] Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

TABLE VII.
Effect of Progressively Increasing Concentrations of Ox Bile Salts* on Coagulation Time of Normal Plasma in the Presence of an Optimum Concentration of Rabbit Brain Emulsion.
(Results in one of 3 experiments.)

Concentration of bile salts, mg. %	50	100	150	200	250	300	400	500	600	700
Coagulation Time in Seconds	22	23	24	26	28	30	35	40	65	no clot

*Fairchild Bros. & Foster.

methods are available for the isolation of bile salts from icteric blood.

Prolongation of the coagulation time was observed also in one case of uremia (blood N.P.N. 238 mg. %). The coagulation time was found to be 32 seconds, as compared with 22 seconds for the control.

Table VIII is a summary of the findings given above.

TABLE VIII.
Comparison of Coagulation Times of Plasma in Presence of an Optimum Concentration of Thromboplastic Substance in Various Experimental and Pathologic Conditions.

Experiment	Coagulation Time, Seconds	Probable Mechanism of Action
Normal	22	Increased activation of prothrombin by thromboplastin
Diluted plasma (1)	37	Diminution of prothrombin
Heparinized plasma (2)	37	Antithrombic action of heparin
Plasma with added NaCl (3)	33	Interference with fibrin formation
Uremic plasma (4)	32	Mechanism not proven
Icteric " (5)	35	
Plasma with added $CaCl_2$ (6)	35	
Plasma with added ox bile salts (7)	37	

(1) 40% plasma and 60% 0.85% NaCl.
(2) 1 unit Heparin (Connaught Lab.) per cc. plasma.
(3) 1.7% NaCl added.
(4) N.P.N. 238 mg.%.
(5) Icterus index 110.
(6) 0.35% $CaCl_2$ in 1.2% NaCl. Total normality of chlorides 0.35.
(7) 400 mg.% ox bile salts (Fairchild Bros. & Foster).

Summary. The addition of various substances to normal plasma caused a prolongation of the coagulation time as measured by the Quick reaction. Therefore prolonged coagulation time by this method does not always indicate a deficiency of prothrombin in the plasma.

9722 P

Canine, Murine, and Human Leptospirosis in California.

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In March, 1936, Dr. S. T. Michael of the hospital of the Society for the Prevention of Cruelty to Animals solicited assistance in the diagnosis of a highly fatal malady which affects with increasing frequency the older dog population of San Francisco and vicinity. Unfortunately, only dead animals or dogs in a moribund state were available until early in 1937. The examinations were consequently limited to autopsies, search of the tissues stained by the silver method, and attempts to isolate from the heavily contaminated organs by guinea pig passages, the leptospira readily demonstrable in dark field preparations of the kidneys, the spleen and occasionally the blood. The postmortem findings made on 67 dogs arranged themselves into 2 groups: the *Icteric Type* (24 females and 9 males), which corresponds to the so-called Weil's disease or "yellows" or canine jaundice observed in England by Okell, Dalling and Pugh,[1] and in Holland by Klarenbeek,[2] and the hemorrhagic type (27 females and 9 males) resembles greatly the Stuttgart disease or dog typhus.[3] Although leptospiras have been demonstrated in the kidneys and occasionally spleen, liver and blood, both in dark field preparations and in properly stained sections prepared from cadavers belonging to both types, cultures have only been obtained directly from the blood of 6 dogs which were definitely jaundiced. Contrary to the impressions conveyed by published reports, the isolation of these spirochaetes was connected with great difficulties since the organisms produced transitory non-fatal infections in very young guinea pigs. On passage the infectiousness was occasionally enhanced but the rodents rarely died, and then in exceptional instances with icterus or hemorrhages. One additional strain was secured in this manner. Since neither the original tissues from 40 dogs nor several of the pure cultures infected white mice and rats, it was suspected that the leptospiras were probably different from the classical *Leptospira icterohaemorrhagica.* They were sent to Prof. W. A. P. Schüffner of the Section of Tropical Hygiene in the Royal Colonial Institute, Amsterdam, for a comparative study. He very kindly reported that the strains are identical with *Leptospira canicola.* That this type of spirochaete is the causative agent of the dog disease and probably widely distributed is further proven by the positive serum reactions (1:10,000) in a recovered dog from Northern California, and of a police dog (1:300) with renal lesions remarkably similar to those described by Dhont, Klarenbeek, Schüffner and Voet.[4] The California canicola leptospirosis is complicated by icterus and in this respect differs from that seen in Holland. It is repeatedly noted in the Dutch publications that jaundice is not observed in the *Leptospira canicola* infections of dogs or human beings who accidentally contract this spirochaetosis.

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[1] Okell, Dalling, and Pugh, *Vet. J.*, 1925, **81**, 3; *Brit. Med. J.*, 1925, Feb. 7, 266; *Proc. Roy. Soc. Med.*, 1925, **18**, 17.

[2] Klarenbeek, *Tijdschr. v. Diergeneesk.*, 1935, **62**, 1182; *Twelfth Int. Vet. Congress, New York*, 1934, **3**, 349.

[3] Wirth, D., *Tierärztl. Rundschau*, 1935, **41**, 609; Lukes, J., *Prag. Arch. f. Tiermed. u. vergleich. Pathol.*, 1926, **6**, 233.

The history of a veterinarian, who infected himself while caring for dogs with leptospirosis and whose blood agglutinated in the eighth month after recovery *Leptospira canicola* in a dilution of 1:300 and *Leptospira icterohaemorrhagica* in a dilution of 1:30 (Prof. W. A. P. Schüffner), records a definite icterus and nephritis. According to Schüffner and Walch-Sorgdrager[5] and Esseveld,[6] *Leptospira canicola* infections may escape detection, since they are not complicated by icterus and masquerade occasionally with meningeal signs as a malady clinically distinct from Weil's disease. The experiences in Holland leave no doubt that the *Leptospira canicola* infections of the dog are frequently followed by a chronic nephritis accompanied by a urinary shedder state. With a relatively wide distribution of this canine disease transmissions to man may be anticipated.

Classical Weil's disease with jaundice in sewer workers and a gardener has been diagnosed in San Francisco with the aid of the usual serological methods. The sera specifically agglutinated or lyzed *Leptospira icterohaemorrhagica.* Since the source of this type of leptospirosis is invariably the rat, surveys as to the extent of murine renal spirochaetal infections have been made with the assistance of the San Francisco Department of Public Health and the laboratory for plague suppressive measures, U. S. Public Health

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[4] Dhont, Klarenbeek, Schüffner, and Voet, *Nederl. Tijdschr. v. Geneesk.*, 1934, **78**, 5197.

[5] Schüffner and Walch-Sorgdrager, *Bull. de l'Office internat. d'hyg. pub.*, 1937, **29**, 297.

[6] Esseveld, Thesis, Amsterdam, 1937.

Service. At random examinations of the kidneys of 420 rats *(Mus norvegicus, M. alexandrinus* and *M. rattus)* in dark field preparations yielded positive leptospira findings in 139, or 33%. In the vicinity of a stable, 42 of 44 old Norvegicus rats have been found infected. Twenty-two strains have been isolated by guinea pig passage. They all produced fatal infections with icterus on the first, second or third passage. Both the pathological effect and the serological tests in part conducted by Prof. W. A. P. Schüffner identify the rat spirochaetes as *Leptospira icterohaemorrhagica*. Since these preliminary observations have thus far failed to demonstrate *Leptospira canicola* in the rat population, one is justified in concluding that the host relationship of the two spirochaetes recognized in California is the same as in Holland. It has been repeatedly emphasized by Schüffner and Klarenbeek that the dog, the only reservoir for *Leptospira canicola,* causes aberrant human infections. On the other hand, the rat reservoir of *Leptospira icterohaemorrhagica* is equally the source of infections for both man and dog. Canine Weil's disease due to *Leptospira icterohaemorrhagica*, which spreads independently of exposure to rats by simple contact from dog to dog, has not as yet been identified in the United States.

9723 P

Effect of Prontosil on Blood Cells.

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Despite the now voluminous literature on sulfanilamide and prontosil, little has been recorded on the cytological changes in blood or tissues taken from animals given doses of this drug over a prolonged period of time. In order to evaluate the tissue changes following administration of prontosil, the following experiment was undertaken with a desire to demonstrate the cytological changes in parenchymatous organs during the administration of moderate doses of the drug, and to follow the changes in the cellular elements of the blood throughout the experiment.

For this purpose, 12 rabbits were chosen of equal size and age, weighing 1200 gm. apiece. These were mixed males and females which were kept in well-aired, clean cages during the entire experiment and fed on a normal laboratory diet of rolled oats, alfalfa, fresh green vegetables, and cod liver oil. They were divided into 2 groups, 4 animals being kept as controls and 8 animals were given 10 cc. (0.25 gm.) of prontosil (Winthrop Chemical Company) per kilogram of body weight for 21 consecutive days. The injections were given alternately in either hindleg in the adductor muscle group. This dosage was chosen because it represents an amount slightly in excess of that advised by Long[1] for clinical use. The injections apparently caused no pain and the drug was absorbed very rapidly from the muscle. In 2 to 3 minutes the ears would show a pink coloration and after the first injection, the urine was tinged red by the drug administration. At no time during the experiment did any evidence of pain, edema, soreness or infection develop in the areas of injection.

The color index of the erythrocytes, the erythrocyte count, the hemoglobin percentage, the white blood count with differential studies and the platelet count were done daily throughout the experiment on all animals. At the end of 21 days, the animals were killed by the intravenous injection of 10 cc. of air and necropsied. Tissue sections were made.

Throughout the 21-day period the erythrocyte, polymorphonuclear, and platelet counts ran closely parallel in the treated and untreated animals. The erythrocytes varied between 5 and 6 million, the platelets from 4 to 7 hundred thousand, and the white blood cells from 6 to 10 thousand per cubic millimeter. The hemoglobin level in both groups remained in a narrow zone between 60 and 70%. In the rabbits receiving treatment there was a rise of from 10 to 15% in the number of erythrocytes and in the hemoglobin concentration in the week following the 21-day period of injection. The other elements remained unchanged during this period.

In the differential studies, the treated animals showed an increase of from 10 to 14% of eosinophiles in the peripheral blood over the normally high level seen in rabbits, and in the second week of injection of the drug, increased numbers of stippled erythrocytes were found.

Microscopic examination of the tissues showed a moderate congestion of the splenic pulp with erythrocytes, and an increase in the proportion of single-lobed eosinophiles in the bone marrow.

Conclusions. 1. Prontosil given to rabbits in a dosage of 0.25 gm. per kilo of body weight causes no numerical change in the cellular elements of the blood in 21 days. 2. An increase in stippled erythrocytes and eosinophilic polymorphonuclear cells occurs, suggesting mild bone marrow depression. 3. The parenchymatous organs show congestion of the spleen and an increase of eosinophiles in the bone marrow, suggesting again an early bone marrow depression.

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[1] Long, P. H., and Bliss, E. A., *J. A. M. A.*, 1937, **108**, 32.

9724 P

Effect of Theelin on Transplantable Mammary Rat Adenofibroma.*

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Murray,[1] Lacassagne[2] and others have shown in males of certain strains of mice that estrogenic hormone may awaken a dormant malignant potentiality in breast tissue. Lewis and Geschickter[3] reported the presence of measurable amounts of estrogenic and gonadotropic hormones in adenofibromas of the human female breast. Numerous investigators have inquired into this phenomenon in relation to other mammalia and contradictory evidence has been forthcoming. Recently Frank and his coworkers[4] reported observations throwing doubt on the influence of these hormones on breast tissue, for other tissues also contain large amounts of estrogenic hormone normally. Heiman and Krehbiel[5] found measurable amounts of estrin in rat adenofibromas, but Mohs[6] not only failed to find appreciable amounts in such tissue but also observed that estrin administration raised the estrin content in these tumors only if high doses of this hormone were given 2 or 3 days prior to sacrifice.

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* Aided by grants from the Rockefeller Fluid Research Fund of Stanford University School of Medicine, and from the Scientific Research Committee of the American Medical Association. We are grateful to Parke, Davis and Company for the donation of theelin used in this experiment.

[1] Murray, W. S., *J. Cancer Research*, 1928, **12**, 18.

[2] Lacassagne, A., *Compt. rend. Acad. d. sc.*, 1932, **195**, 630.

[3] Lewis, D., and Geschickter, C. F., *J. A. M. A.*, 1934, **103**, 1212.

[4] Frank, R. T., *et al.*, Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1665.

[5] Heiman, J., and Krehbiel, O. F., *Am. J. Cancer*, 1936, **27**, 450.

[6] Mohs, F. E., *Am. J. Cancer*, 1937, **29**, 356.

Since we have access to several strains of transplantable mammary adenofibromas having marked malignant potentialities, as shown by the development of various strains of extremely malignant sarcomas, we have undertaken a series of experiments to study the influence of long-continued injections of theelin on the various strains of our adenofibromas normally containing only very small amounts of measurable estrogenic hormone. In the present experiment male and female white rats from 135 to 150 days of age implanted with a fast-growing adenofibroma of strain 5-B I were injected subcutaneously daily with either 100 or 200 i.u. of aqueous theelin. Thirty-two animals were subdivided into 3 groups. One group, consisting of 6 males and 5 females received 100 i.u. of theelin and a second similar group was given 200 i.u. of theelin over a period of 77 days, while the third group of 5 males and 5 females was used as a control. During the period of injection tumors were measured every other day and at the termination all animals were sacrificed and all tumors were studied microscopically.

Ordinarily the adenofibroma 5-B 1 used in this experiment produces a very glandular tumor. However, in this transplantation the tumors tended toward fibromas with a definite reduction of adenomatous material. Failure of transplant "take" occurred with about equal frequency in each group—2 in the controls and 3 in each of the treated groups. The males grew more glandular tissue than the females in all 3 groups. As compared with male or female animals in the control group, theelin-injected animals showed no increase in gland tissue. Also, the glandular response was not beyond that expected from long-continued injection of an estrogenic hormone. There was no unusual tendency towards sarcomatous degeneration in any of the tumors. No appreciable difference in the organ weights occurred in the different groups except for a slight reduction in adrenal weights in both theelin groups. The estimation of estrin content will be reported elsewhere.

TABLE I.
Mean Daily Changes in Body Weight (gm./day).
Group I, Control vs. 100 i.u. theelin; Group II, Control vs. 200 i.u. theelin; Group III, 100 i.u. theelin vs. 200 i.u. theelin; Group IV, Control vs. individuals receiving 100 i.u. plus those receiving 200 i.u. theelin.

Group	Control	100 i.u. theelin		200 i.u. theelin	Diff. (d)	n*	t*	P* for abs. val. of (d)	P for (—d)
I	+.288	+.123			—.165	19	2.227	.040	.020
II	+.288			+.054	—.234	19	2.317	.035	.018
III		+.123		+.054	—.069	20	0.850	.400	.200
IV	+.288		+.090†		—.198	30	2.598	.015	.008

TABLE II.
Mean Daily Changes in Tumor Weight (gm./day).
Groups: As in Table I.

<table>
<tr><th>Group</th><th>Control</th><th>100 i.u. theelin</th><th>200 i.u. theelin</th><th>Diff. (d)</th><th>n*</th><th>t*</th><th>P* for abs. val. of (d)</th></tr>
<tr><td>I</td><td>+.218</td><td>+.029</td><td></td><td>—.189</td><td>14</td><td>1.808</td><td>.093</td></tr>
<tr><td>II</td><td>+.218</td><td></td><td>+.057</td><td>—.161</td><td>14</td><td>2.996</td><td>.163</td></tr>
<tr><td>III</td><td></td><td>+.029</td><td>+.057</td><td>+.028</td><td>14</td><td>0.728</td><td>.480</td></tr>
<tr><td>IV</td><td>+.218</td><td colspan="2">+.043†</td><td>—.175</td><td>22</td><td>0.995</td><td>.333</td></tr>
</table>

*n = "degrees of freedom."

t = difference of means/standard error of that difference, adjusted for "n."

P = probability of a difference as great or greater than that obtained, occurring by chance alone. See Fisher, R. A., "Statistical Methods for Research Workers," London, Oliver & Boyd, 1934, 5th Ed., Chap. V.

† It is proper to combine these into one group as there is no significant difference between the administration of 100 i.u. and 200 i.u.

Conclusions. In spite of long-continued injections of theelin, a rapidly growing mammary adenofibroma of the white rat transplanted into 32 rats lost a large part of its adenomatous content. No unusual growth behavior attributable to the injection of an estrogenic hormone could be demonstrated in the tumor tissue either grossly or microscopically. Theelin decreases body weight, but the size of the dose, in the limits given, is not significant. In other words, the loss in weight is not significantly greater when administering 200 i.u. than it is when administering 100 i.u. However, theelin has no significant effect on the mean daily changes in tumor weight.

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Filtrability of the Chinese Strain of *Spirochaeta recurrentis.*

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Novy and Knapp[1] first demonstrated that after having gone through Berkefeld filters, the filtrate of blood from animals infected with the American strain of relapsing fever spirochaetes was infectious; these workers concluded that the form present in the filtrate represented an invisible developmental stage. Later Breinl and Kinghorn[2] working with *Sp. duttoni* arrived at the same conclusion. Todd and Wolbach[3] reported that *Sp. duttoni, Sp. kochi,* and 2 other strains of African tick-borne relapsing fever spirochaetes could be forced through a "V", "N" or "W" Berkefeld filter under a pressure of 50 lb. to the square inch, but not under atmospheric pressure. These two authors, however, did not examine the filtrate under the microscope for spirochaetes, but based their conclusion on the results of animal inoculations with the filtrates. In regard to *Sp. obermeieri* Todd[4] stated that in its typical form it can be forced through a "W" filter. The present report presents the results of studies on the filtrability of the Chinese strain of *Spirochaeta recurrentis.*

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[1] Novy, F. G., and Knapp, R. F., *J. Infect. Dis.*, 1906, **3**, 291.

[2] Breinl, A., and Kinghorn, A., *Memoir XXI, Liverpool School of Tropical Medicine,* September 1, 1906.

Material and Method. Bacteria-proof Berkefeld filters "V", "N", and "W", were used. The patency of the filters was tested in every experiment by mixing the material to be filtered with pure cultures of *B. dysenteriæ* Shiga, and *B. typhosus* and by culturing the filtrate for these organisms. In all instances the filtrate was sterile. Altogether the spirochaetes from 6 Chinese patients suffering from relapsing fever have been studied. The blood was taken during fever and contained in every instance more than 10 spirochaetes per oil-immersion darkfield. Only plasma was needed. It was obtained as follows: To a 250 cc. sterile Ehrlenmeyer flask containing 10 cc. sodium citrate glucose mixture (10% sodium citrate and 10% glucose) and 40 cc. normal saline solution, 80-100 cc. blood freshly obtained from a relapsing fever patient was added. The flask was gently rotated a few times and then allowed to stand for 2 hours or so in order to obtain about 80 to 100 cc. plasma. After eliminating the blood cells, the number of the spirochaetes present in the plasma is almost double that present in whole blood, provided there is no anemia.

As it is very difficult to filter plasma under atmospheric pressure, filtration was facilitated by a special suction pump. The pressure applied varied from 5 to 25 lb. The duration of suction or filtration varied from 10 to 40 minutes.

Only specimens of plasma containing numerous spirochaetes were used. The filtrate was centrifuged at high speed for half an hour in an ice-cup. A drop of the "filtrate sediment" was obtained by means of a fine capillary pipette and examined under the darkfield microscope for spirochaetes. At least 4 drops of the "filtrate sediment" were examined for not less than half an hour before the result was declared negative. In most instances the filtrate was also inoculated into splenectomized but otherwise normal squirrels, all

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[3] Todd, J. L., and Wolbach, S. B., *J. Med. Res.*, 1914, **30**, 27.

[4] Todd, J. L., *Parasit.*, 1920, **6**, 152.

TABLE I.

Table Showing Filtrability of Chinese Strain of *Sp. recurrentis*.

Exp. No.	Pt.'s. hosp. No.	Pt's. temp., °C	Duration of illness, days	Berkefeld filter	Pressure applied, lbs./sq. in.	Suction applied min.	Darkfield examination of filtrate	Squirrel inoculation		
								Filtrate cc.	Squirrel No.	Result
1	53528	40.2	18	V	5-10	20	+			
				N	20-25	20	0			
2	53571	40.0	3	V	20-25	10	+	0.5 I.P.	163	+++
				N	20-25	20	0			
				W	20-25	20	0			
3	54017	39.4	9	V	5	15	+ occ.	3 I.V.	182	+++
				N	5-10	20	0			
4	54155	39.4	30	V	5-10	15	+	1 I.V.	172	+++
				N	5-10	15	+ occ.			
				W	5-10	15	0	2 I.V	197	+++
5	55162	38	32	N	5-10	20	+ occ.	3 I.P. 2 I.V.	228	+++
6	55218	39.6	5	V	5	15	+ occ.	5 I.P.	230	+++
				N	5	40	0	5 I.P.	231	0

occ. = spirochætes occasionally present. I.P. = intraperitoneal. I.V. = intravenous.

but one of which subsequently developed relapsing fever and showed spirochaetes in the blood.

The results of the experiments are summarized in Table I. It is clear that under a pressure of 5 to 10 lb. to the square inch, a number of spirochaetes in some form pass through Berkefeld filters "V", "N',' and "W". Spirochaetes were easily found in all the "V" filtrates. but only occasionally in one of the "N" filtrates. The "W" filtrates showed no spirochaetes under the darkfield microscope, but the filtrate caused relapsing fever when inoculated into a squirrel.

The fact that the "W" filtrates and most of the "N" filtrates failed to show spirochaetes under the darkfield microscope does not necessarily mean that these filtrates actually contained absolutely no spirochaetes in their normal form, for it remains possible that the spirochaetes present were so scanty that they were missed when looked for under the microscope. Hence, the fact that such a filtrate caused relapsing fever when inoculated into a splenectomized squirrel does not prove that the spirochaetes were present in an invisible or granular form. Further, since spirochaetes in their usual form have been shown to pass through Berkefeld filters, more direct evidence than has been offered is needed to establish the existence of an invisible or granular form.

Conclusion. The Chinese strain of *Sp. recurrentis* passes through Berkefeld filters "V", "N", and "W" under a pressure of 5 to 10 lb. to the square inch.

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Susceptibility of Rodents *(Myospalax fontanieri)* to Experimental Infection with Rickettsiae of Chinese Typhus.

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In general, guinea pigs have served satisfactorily as the experimental animal in the study of rickettsial diseases. However, when dealing with the European type of typhus fever, which occurs locally, these animals presented certain disadvantages. The only clinical manifestation of this disease in guinea pigs is a more or less typical period of fever following an average interval of incubation. With the possible exception of the finding of Durand,[1] who used a special technique, their blood serum has not been found to agglutinate proteus X_{19}. So unless one uses the rarely obtainable rickettsial emulsion as the antigen, there can be no positive serological evidence of this infection. The study of histological lesions would necessitate the sacrifice of the animals which is often undesirable. Rabbits have been used to obtain a positive Weil-Felix reaction, but even then, the titre is usually low and is, therefore, often inconclusive. Rats give only an inapparent infection. Recently the ground squirrel or "Zieselmaus" has been independently[2, 3] found to be extremely susceptible to the European type of typhus. As many as 70% of the animals infected die from this disease. However, the blood serum of this animal also failed to give a positive Weil-Felix reaction. It was for the purpose of finding another laboratory animal in which these difficulties might be circumvented that led us to make a study of a local species of rodent *(Myospalax fontanieri)*, Milne-Edwards; the following is a brief report of our results.

Adult animals, weighing 200 to 300 gm., were used. A preliminary observation period of 7 to 10 days was made before actual use in order to note the effect of confinement on these untamed animals. At the end of this period, each animal received either subcutaneous, or more often, intraperitoneal injections of one-fifteenth of the brain of a guinea pig infected with a passage strain of typhus. The body temperature was taken daily and recorded. It may be mentioned that the normal body temperature of these rodents varies from 36.6°-37.5°C.; in the present study any temperature reading above 38°C. was taken to indicate fever. Animals were bled for blood culture and for the Weil-Felix reaction a few days after they showed fever. It is to be noted that normal animals gave consistently negative Weil-Felix reactions. Some of them were also sacrificed for pathological examination and for reinoculation into guinea pigs.

Altogether 28 animals were used for inoculation with rickettsial material from guinea pigs. Among these, 5 died of secondary infection within one week after inoculation, and these were, therefore, discarded. Among the remaining 23 animals, 16 showed varying periods of fever, preceded by an incubation of 5-12 days, with an average of 7 days (7 animals). The Weil-Felix reaction was done on 19 of these animals with 12 positive and 7 negative results. The titre ranged from 1:20 or 1:40 (2 animals) to 1:160 or above (7 animals). The highest titre was 1:640 taken on the fifth day of fever. Ten died during the febrile attack; in some of these animals the serological reaction was positive. Typhus nodules appeared irregularly in the brains of these animals. Of 8 examined, only 2 gave positive findings, one with an extensive distribution, the other with few lesions. A small amount of the brain was reinoculated into guinea pigs and in every case examined, a typical typhus reaction occurred. Of special interest is one animal which showed no fever for a period of 16 days, but gave a positive Weil-Felix reaction with a titre of 1:80; a small amount of an emulsion of its brain produced a typical typhus reaction after inoculation into guinea pigs. Serial passages in the rodents were attempted but owing to the lack of further supply of the animals at that time, only 2 passages were made. Animals in both generations showed regular fever, and a small amount of the brain of one of the third generation produced a typical typhus reaction in guinea pigs.

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[1] Durand, R., *Compt. rend. Soc. de biol.*, 1934, **116**, 360.

[2] Jelin, W., and Grossmann, J., *Arch. f. Schiff. u. Trop. Hyg.*, 1933, **37**, 346.

[3] Serebrennaji, A. I., Grünfeld, A. A., and Neumann, M. W., *Zentralb. f. Bakt.*, 1933, **1**, Orig. 129, 254.

Rodents found locally and of convenient size were shown to be susceptible to infection with local typhus rickettsiae. They showed not only fever after an incubation period of about 7 to 11 days, but the sera of the majority also gave positive Weil-Felix reactions in titres ranging from 1:20 to 1:160 and above which are considered to be significant. Typhus nodules, however, were found only in 2 of the 8 brains examined. On reinoculation to guinea pigs, the brain of these animals, which included one animal that did not show febrile reaction, invariably gave rise to a typical typhus reaction. It, therefore, appears clear that we have an animal which may prove more advantageous than the guinea pig for the experimental study of typhus.

9727

In vitro Photodynamic Action of Methylene Blue on *Trypanosoma brucei.*

T. T'ung. (Introduced by S. H. Zia.)

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In reviewing the literature dealing with the *in vitro* photodynamic action of acridine dyes on microörganisms, one is impressed with the fact that adequate study of this action on protozoa has yet to be made. The few reports in existence deal almost exclusively with the effect of the photodynamic action of various dyes on the motility of a few protozoa. Such were the studies made with *Leishmania donovani* flagellates by Noguchi[1] and those of trypalavine on *Trypanosoma brucei* by Tappeiner,[2] Bruynoghi,[3] and Jancso.[4] It seems plausible that motility alone cannot be taken as the criterion of life or death of the treated organisms. In addition, it is not inconceivable that such action might bring about some morphological changes on the treated protozoa. With these views in mind, it was considered of interest to restudy the *in vitro* photodynamic action on *Trypanosoma brucei* with methylene blue, a dye which hitherto has not been tried on this organism.

Citrated blood of guinea pigs infected with *Trypanosoma brucei* was diluted with normal saline. After the cells had been removed by centrifugation at low speed, the supernatant fluid which contained the organisms was mixed with various dilutions of saturated solution of methylene blue in the ratio of 9 to 1 respectively. Part of the mixture contained in a Petri dish was put over a Frigidaire cooling machine and exposed to a 100-Watt lamp at a distance of 10 cm. for one hour. The other portion of the mixture was kept unexposed as control. The motility of the exposed and unexposed *Trypanosoma brucei* was examined at the end of 30 and 60 minutes respectively. After exposing for one hour, 0.5 cc. of each of the exposed and unexposed mixtures were injected intraperitoneally into a series of white mice, 4 in each group. Daily examination for the presence of trypanosomes in the blood of the inoculated animals was made for 3 weeks. Part of the mixtures was kept at 37°C. and was examined from time to time to observe any change in morphology as stained with the Wright stain.

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[1] Noguchi, H., *Proc. International Conference on Health Problems in Tropical America, Kingston, Jamaica*, 1924, p. 455.

[2] von Tappeiner, H., *Erg. Physiol.*, 1909, **8**, 698.

[3] Bruynoche, par R., *Compt. rend. Soc. de Biol.*, 1932, **110**, 124.

[4] von Jancso, N., *Zentralb. f. Bakt.*, Original 1931, **122**, 388; 1932, **123**, 129.

The following results were obtained: (a) Methylene blue, up to dilution of 1:100,000, in the presence of light, was sufficient to immobilize *Trypanosoma brucei*, whereas, without light this dye could only exert a similar effect in a concentration of 1:100. (b) Sixty minutes of exposure yielded results similar to a 30-minute exposure. (c) The immobilizing effect of the dye was parallel to its lethal activity. (d) All the mixtures containing immobilized trypanosomes failed to produce infection in white mice. Furthermore animals infected with trypanosomes treated with the photodynamic action of methylene blue in a dilution insufficient to immobilize the organisms were found to have a longer incubation period and survival than the control ones.

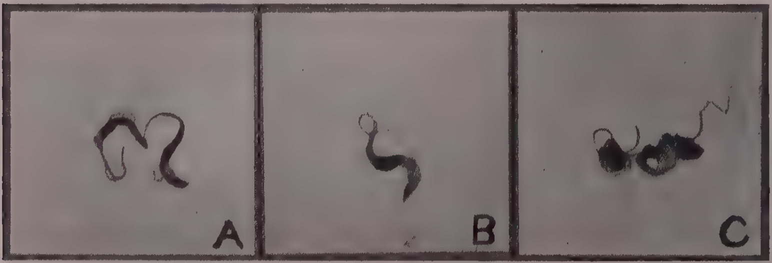

FIG. 1.
Morphological appearance of *Trypanosoma brucei* one week after treatment with various methods.
A. Inactivated by the photodynamic action of methylene blue.
B. Treated with methylene blue alone.
C. Preserved with 0.4% formalin.

As it has been previously shown that the photodynamic action of methylene blue exerts a preservative effect on pneumococci[5] the same preservative effect on the *Trypanosoma brucei* has also been demonstrated. As examined in stained smears, the cellular structure of the photodynamically treated trypanosomes—concentration of 1:100 or 1:1,000 of methylene blue kept at 37°C. for a month was used—is almost comparable to that of the fresh organisms. On the other hand, trypanosomes heated at 56°C. for half an hour lose their cellular integrity. When they were preserved with 0.4% formalin their shape became distorted and they began to show signs of disintegration if kept at 37°C. for 2 weeks. It is also worthy of note that *Trypanosoma brucei*, although equally susceptible to the photodynamic action of eosin, cannot be preserved by this treatment.

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[5] T'ung, T., PROC. SOC. EXP. BIOL. AND MED., 1935, 33, 828.

The fact that *Trypanosoma brucei* can be well preserved morphologically by the photodynamic action of methylene blue even at incubator temperature for a considerable length of time, and that a large quantity of such treated organisms can be easily obtained by the method described, offers the possibility of studying active immunization in susceptible animals with a vaccine. Since the protective property of heat-treated trypanosoma vaccine has not been satisfactorily demonstrated such a method is worthy of further investigation.

9728

Observations on the "Latent Period" in Experimental Diabetes Insipidus.*

Allen D. Keller.

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The "latent period" as designated and described by Fisher, Ingram, and Ranson[1] was encountered by us without exception in upward of 30 experiments in the dog where permanent d.i. of varying severity has followed hypothalamic lesions. The operation was usually followed immediately by a polydipsia and polyuria which was invariably interrupted by a return of the water exchange toward normal, usually reaching to below normal, in a matter of 2 to 6 days. The more or less abrupt onset of the permanent phase of polydipsia and polyuria occurred on the seventh to eleventh postoperative day. Our series differed from the Fisher series in that the d.i. of the temporary phase was, as a rule, greater in magnitude than that of the permanent phase.

In view of the evidence indicating that a functioning pars anterior of the hypophysis is essential for the occurrence of d.i. *in its maximal form*, it seemed possible that the events of the latent period might be explained on the basis of the operative procedure producing in addition to an immediate and lasting impairment of the antidiuretic secretory mechanism also a transitory depression of the pars anterior, the onset of the permanent phase signaling the returning function of this tissue.

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* Aided by a grant from the Rockefeller Foundation.

[1] Fisher, C., Ingram, W. R., and Ranson, S. W., *Arch. Neurol. and Psychiat.*, 1935, **34**, 124.

This explanation seemed at first particularly possible because the essential characteristics of the latent period have been duplicated in a series of dogs in which total hypophysectomies were attempted and, after a lapse of several days, anterior pituitary extract was administered (see upper graph, Fig. 1). There occurred in these dogs on the first day after operation a striking (500 cc. per kilo) though moderate d.i. which on succeeding days became progressively reduced either to normal (approx. 50 cc.) or to a level intermediate between normal and that of the immediate transitory phase (200 cc.). (If the spontaneous consumption of water and food is pre-

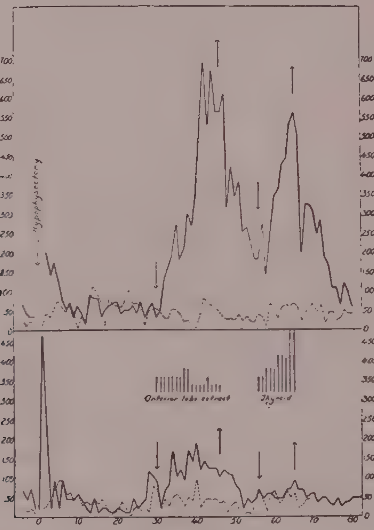

FIG. 1.

Solid line—water intake. Stippled line—food intake. Ordinate—cc. and gr. per kilo. body weight. Abscissa—days after operation. Upper curves—dog 192, wt. 5.5 kilo. Lower curves—dog 193, wt. 4.2 kilo. The magnitude of the polydipsia on the first day in 192 was not determined because of its water being contaminated with feces. Both dogs were operated the same day and received the same dosage of anterior pituitary extract (.5 to 1.5 cc. per day subcutaneously) and thyroid (1 to 4 gm. U.S.P. powder per day by capsule) irrespective of body weight. In dog 192 there was no gross involvement of the hypothalamus but the hypophysectomy was incomplete in that fragmentary islets of tuberalis cells remained attached to the hypothalamus and a remnant of the pars intermedius remained in the base of the pituitary fossa associated with the posterior lobe artery. In dog 193 the larger portion of the tissue of the hypophyseal stalk remained intact as well as remnants of pars intermedius and anterior.

vented as by prolonged anesthesia or a very sore head, this transitory phase may not be detected.) Whether or not the water exchange returned to normal or leveled off above normal, a subsequent daily administration of anterior pituitary extract over a period of several days elicited characteristically a striking d.i. (see upper curve, Fig. 1).

The return of the water exchange to normal following an attempted total hypophysectomy is indicative, we believe, that a functional remnant of antidiuretic secretory tissue remains intact. This belief is based on our findings in a group of dogs in which only partial hypophysectomy was performed in that the hypophyseal stalk was purposely left attached to the brain.† In these animals there was also usually an immediate transitory d.i. after which the water exchange always returned quickly to normal or below and in addition the preparations were subsequently resistant to anterior pituitary extract. (See lower curves, Fig. 1.) By taking special care in severing the body of the gland, previous to its removal, from the stalk as by a clean cut with scissors the immediate transitory d.i. has in instances been avoided. We interpret the immediate transitory d.i., when it appears in such an animal, as being due to a temporary traumatic paralysis of the secretory tissue of the stalk. The fact that such a preparation can be prepared without transitory d.i. occurring would seem to indicate that this tissue normally secretes the antidiuretic principle in the intact animal; that is, there is no reason to believe that it assumes this function after the operation.[2]

Assuming that there is a complete absence of the antidiuretic principle in the presence of *total* hypophysectomy, the immediate[3] transitory d.i. which follows this procedure may well be explained in terms of the evidence that d.i., at least in its maximal form, is dependent upon thyroid activity and on the basis of the known difference in latencies of action of the antidiuretic and thyroid substances. Thus one would expect in the presence of a simultaneous abrupt removal of the antidiuretic and the thyrotropic principles that the effects of the former would appear before the latter. The pars anterior deprivation effect in this respect seems, however, already evident on the first day after operation because the magnitude of the water exchange on this day in these experiments has never approached that encountered following hypothalamic lesions. On the basis of our observation we suspect that in the situation of a complete absence of the antidiuretic principle in the presence of an intact pars anterior that the water exchange would be a liter or more per kilo.[3b] Again the presence of a persistent submaximal d.i. in the total absence of the pars anterior is consistent either with the view that d.i. is wholly dependent upon thyroid activity[4] or with the possibility that a low grade d.i. is possible in the total absence of thyroid tissue,[5] thyroid activity being essential only for the occurrence of d.i. in its maximal form. Even if d.i. is entirely dependent upon thyroid activity, one would expect a mild or moderate form of d.i. to persist following total hypophysectomy because it is known that removal of the pars anterior results in marked hypothyroidism but not athyroidism.[6]

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† This procedure in the dog is equivalent to "hypophysectomy" as it has been practiced in the rat as well as probably in most instances on record for other species.

[2] Sato, G., *Arch. f. exp. Path. u. Pharm.*, 1938, **131**, 45.

[3] *a.* Howet, F., *C. R. de la Soc. de Biol.*, 1936, 798; *b.* Richter, C. P., *A. R. N. M. D.*, 1938, **17**, 392.

The possibility that the events of the latent period are due to a transitory depression of anterior pituitary functions is immediately questioned when it is recognized that a *permanent,* but submaximal, d.i. does occur in the complete absence of the pars anterior.[7] This being true, even in the complete absence of the antidiuretic principle as well as the pars anterior, the lowest level that the water exchange could reach (unless other factors entered) would be considerably above normal. Actually in our series the water intake was frequently reduced to below normal, even to zero, for a day or two just prior to the onset of the permanent phase.

In order to test this possibility in a more specific way, anterior pituitary extract of established potency and dosage[8] was administered daily during the immediate postoperative period to 8 dogs in which it was deliberately attempted to precipitate d.i. by hypothalamic lesions. An immediate moderate, though well marked, d.i. developed in 5 and a mild d.i. in 2 of these dogs. Without exception there was a transitory return of the water exchange to or near to normal, there being no detectable gross change in the characteristics of the latent period. The experiment on dog 215 is typical of the series. Its essential features are graphed in Fig. 2.

Conclusion. In the light of the foregoing analysis it seems that

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[4] *a.* Barnes, B. O., Regan, J. F., and Buano, J. G., *Am. J. Physiol.,* 1933, **105**, 559; *b.* Mahoney, W., and Sheehan, O., *Am. J. Physiol.,* 1933, **112**, 250.

[5] *a.* Fisher, G., and Ingram, W. R., *Arch. Int. Med.,* 1936, **58**, 117; *b.* Findley, T., An. Int. Med., 1937, **11**, 701.

[6] Houssay, B. A., *Endocrinol.,* 1934, **18**, 409.

[7] *a.* Reichert, F. L., and Dandy, W. E., *Bull. Johns Hopkins Hosp.,* 1936, **58**, 418; *b.* Mahoney, W., and Sheehan, D., *Brain,* 1936, **59**, 61.

[8] Keller, A. D., PROC. SOC. EXP. BIOL. AND MED.,1937, **36**, 787.

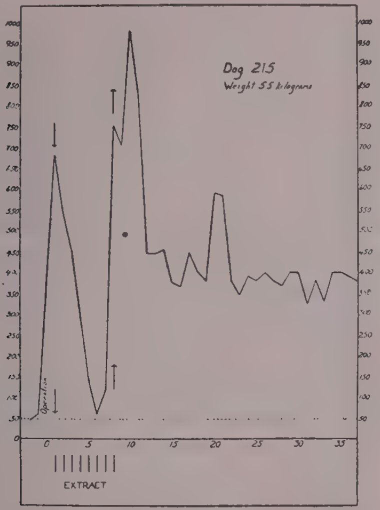

FIG. 2.

Food and water intake as in Fig. 1. Anterior pituitary extract 1 cc. daily subcutaneously. Note that in this experiment the food consumption (Baloration) was maintained constant.

the early temporary alleviation of d.i. which invariably occurs when prolonged d.i. is precipitated by hypothalamic lesions is not due to a fleeting depression of anterior pituitary function.‡

9729 P

Total Creatinine, Phosphates, Calcium and Potassium in Normal and Infarcted Myocardium of the Dog.*

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In 1934 and 1935 with the help of Decherd, the authors studied the mobilization of creatine in experimental myocardial damage[1] and the creatine and glycogen content of normal and infarcted heart muscle of the dog[2] and noted very definite and at times striking changes. The present series of experimental ligations of branches of the left coronary artery of the dog were carried out for the purpose of studying the effect of obstruction of the blood flow upon the phosphates, calcium, potassium as well as creatine levels of the dog's heart muscle.

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‡ We are indebted to Dr. Oliver Kamm of Parke, Davis & Co. for the generous supply of ''anterior pituitary total extract'' which we used in these studies. Although it is now going on the second year since the lot was prepared, it is still satisfactory in potency, as evidenced by experiments executed at the present time (December 1).

* This study was made possible by Grant No. 444 of the American Medical Association.

[1] Herrmann, G., and Decherd, G., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 477.

Twenty out of 24 experiments were satisfactory. The dogs were completely anesthetized with amytal. A tracheal cannula was sewed into place and the chest opened under air pressure. The pericardium was brought up, split open and anchored to the chest wall. The anterior descending branch of the left coronary artery was tied off just below its first visible branch. The dogs were sacrificed after varying intervals from one to 22 hours. Sections were taken from the left ventricular muscle outside and in the infarcted area. Section A of apparently normal left ventricular muscle and Sections B from the infarcted areas were minced and ground up with chemically clean sea sand. Both were extracted with 5% trichloracetic acid and analyzed for total phosphorus, for acid soluble phosphate P and inorganic phosphate P by a modification of

TABLE I.
Averages of Analyses of Sections of the Left Myocardium of 24 Dogs in which a Branch of the Left Coronary Artery Was Ligated.

Aver. in mg. per 100 gm.	TP	PAS	Lipid P	Inorg. P	K
A section (Normal)	285±137	93±9	198±124*	72±11.2	344±110
B section (Infarcted)	258±54	88±13	164±66*	70±7.9	348±111

	Cr (Wet)	Cr (Dry)	Ca	% Solids
A section (Normal)	236±42	1178±190	9.3±1.8	21.15±1.4
B section (Infarcted)	208±61.5	987±300	8.7±2.04	20.25±2.16

TP = total phosphorus.
Lipid P = lipid phosphorus.
Inorg. P = inorganic phosphate P
K = potassium.
Ca = calcium
Cr (Wet) = total creatinine as determined in fresh muscle.
Cr (Dry) = " " " calculated on basis of % solids.
PAS = acid soluble phosphate P
Lipid P values were obtained by subtraction. *Two lost observations account for slight discrepancies in average as subtracted.

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[2] Herrmann, G., and Decherd, G., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1304.

Deniges'[3] method. Other sections were analyzed for potassium by the Brech and Gaebler[4] method, for calcium by Halverson and Bergeim's[5] method, for total creatinine by Folin's method, using the Jaffe reaction. The water content or percentage of solids was determined by drying oven at 110°C. for 24 to 36 hours.[6]

The results as tabulated indicate that the ischemia and anoxemia resulting from ligation of the smaller branches of the anterior descending branch of the left coronary artery produce significant chemical changes. The losses of creatine are accompanied by losses in the total, acid soluble and lipoid phosphates but not of inorganic phosphates or potassium. The creatine losses are less striking than those recorded previously when the main anterior descending branches of the left coronary arteries were ligated[2] due to the smaller and the less definitely demarcated area of infarcted myocardium and almost inevitable inclusion of some normal tissue with the asphyxiated muscle.

9730

Bacteriostatic Action of Sulfanilamide upon Meningococcus in Spinal Fluid.

ERWIN NETER. (Introduced by Ernest Witebsky.)

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It is generally agreed that sulfanilamide and related compounds have a curative effect in natural and experimental infections of man and animals by the hemolytic streptococcus. One of the most interesting features of sulfanilamide as a chemotherapeutic agent is its property to be more or less effective against a wide variety of microörganisms, including the pneumococcus, gonococcus, meningococcus and *Cl. welchii.* The mechanism of the action of sulfanilamide in bacterial infections is not fully understood. There can be no doubt, however, that sulfanilamide exerts a certain degree of bacteriostatic activity *in vitro,* as shown by Bliss and Long[1] Colebrook, Buttle and O'Meara[2]; Colebrook and Kenny[3]; Long and Bliss[4]; Rosenthal,[5] Wengatz, Boak and Carpenter.[6][*] This bacteriostasis may be sufficient to protect leucocytes and to allow the natural defensive macrophages to accumulate (Gay and Clark[7]). Therefore, phagocytosis may be enhanced under treatment with sulfanilamide. It should be kept in mind, however, that no parallelism exists between the effectiveness of sulfanilamide *in vitro* and *in vivo;* Rosenthal[5] could show that sulfanilamide may be more than 100 times as bacteriostatic against pneumococci than against hemolytic streptococci *in vitro,* although this drug is more effective toward the hemolytic streptococcus in animal experiments.

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[3] Deniges, G., *Compt. rend. Acad. Sci.*, 1927, **184**, 330.

[4] Brech, F., and Gaebler, Oliver H., *J. B. C.*, 1930, **87**, 81.

[5] Halverson, J. O., and Bergeim, O., *J. B. C.*, 1917, **32**, 159.

[6] Shohl's modification of Stolte's dry ashing method, Peters, John P., Van Slyke, Donald D., *Quantitative Clinical Chemistry*, Williams & Wilkens Co., Baltimore, 1932, **2**, 70.

Recently it was reported by Branham and Rosenthal[8]; Buttle, Gray and Stephenson[9]; Long and Bliss[10]; McPherson Brown[11]; Proom[12]; Rosenthal, Bauer and Branham,[13] that sulfanilamide has a curative action in experimental meningococcal infections in mice. Clinical observations concerning the treatment of meningococcal meningitis in man were reported by Schwentker, Gelman and Long.[14] In preliminary experiments, Branham[5, 8] demonstrated a bacteriostatic action of sulfanilamide toward the meningococcus *in vitro.* The experiments to be reported here were performed in an endeavor to determine directly the action of sulfanilamide upon the meningococcus present in spinal fluid obtained from patients with meningococcal meningitis. The procedure is based upon the fact that incubation of spinal fluid, which contains only a relatively small number of meningococci, may result in a marked increase in the number of microörganisms. Therefore, the influence of sulfanilamide upon the growth of meningococci in the spinal fluid itself can be examined.

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[1] Bliss, E. A., and Long, P. H., *J. Am. Med. Assn.*, 1937, **109**, 1524.

[2] Colebrook, L., Buttle, G. A. H., and O'Meara, F. A. Q., *Lancet,* 1936, 1323.

[3] Colebrook, L., and Kenny, M., *Lancet,* 1936, 1297.

[4] Long, P. H., and Bliss, E. A., *J. Am. Med. Assn.*, 1937, **108**, 32.

[5] Rosenthal, S. M., *Publ. Health Reports,* 1937, **52**, 192.

[6] Wengatz, H. F., Boak, R. A., and Carpenter, Ch. M., *J. Bact.*, 1938, **35**, 36.

[*] Mellon and Bambas (Mellon, R. R., and Bambas, L. L., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 682) showed that spinal fluid of patients treated with sulfanilamide is bacteriostatic toward the streptococcus.

[7] Gay, F. P., and Clark, A. R., *J. Exp. Med.*, 1937, **66**, 535.

[8] Branham, S. E., and Rosenthal, S. M., *Publ. Health Reports,* 1937, **52**, 685.

[9] Buttle, G. A., Gray, W. R., and Stephenson, D., *Lancet,* 1936, 1286.

[10] Long, P. H., and Bliss, E. A., *J. Bact.*, 1938, **35**, 35.

[11] McPherson Brown, Th., *Bull. Johns Hopkins Hosp.*, 1937, **61**, 272.

[12] Proom, H., *Lancet,* 1937, 16.

[13] Rosenthal, S. M., Bauer, H., and Branham, S. E., *Publ. Health Rep.*, 1937, **52**, 662.

[14] Schwentker, F. F., Gelman, S., and Long, P. H., *J. Am. Med. Assn.*, 1937, **108**, 1407.

The following protocol represents a typical experiment: Four drops of cloudy spinal fluid obtained from a patient with meningococcal meningitis prior to treatment, were mixed with 10 drops of each of the following dilutions of 0.8% sulfanilamide dissolved in physiological saline solution: 1:1; 1:10; 1:100; 1:1000, and 1:10,000 (tubes a-e respectively). The preparation used was *p*-aminophenylsulphonamide, repurified for injection. To the control tube (f) 10 drops of physiological saline solution were added. The experiments were carried out under sterile precautions. The tubes were well shaken and incubated at 37°C. for 48 hours. Films were made at various intervals from all tubes. The result of this experiment is given in Table I.

TABLE I.

Action of P-aminophenylsulphonamide on Growth of Meningococci in Spinal Fluid. 4 drops of spinal fluid of patient with meningococcal meningitis mixed with

	10 drops 0.8% Prontylin diluted with saline solution					10 drops saline solution
	1:1	1:10	1:100	1:1000	1:10,000	
Tubes	a	b	c	d	e	f
Growth after			Growth			
18 hr. at 37°C.	No	No	No	No	Marked	Marked
48 hr. at 37°C.	"	"	"	Some	"	"

It may be seen that sulfanilamide markedly inhibits the growth of meningococcus in spinal fluid. Dilutions of 1:1 (800 mg. %) up to 1:100 (8 mg. %) completely prevented the augmentation of meningococci during 24 to 48 hours; a dilution of 1:1,000 (0.8 mg. %) caused only a partial inhibition. The control tube of spinal fluid mixed with physiological saline solution instead of sulfanilamide showed a very marked increase in the number of meningococci within 24 hours. Similar results were obtained with spinal fluids of 4 more patients.

In order to determine the influence of sulfanilamide upon the viability of meningococci present in spinal fluid, subcultures were made from the spinal fluid-drug mixtures on chocolate ascitic agar plates. These plates were incubated at 37°C. in a jar containing about 10% CO_2. The experiments revealed that no growth or a markedly diminished growth occurred in the spinal fluids previously incubated with sulfanilamide in dilutions of 1:1 (800 mg. %) or 1:10 (80 mg. %) in comparison with spinal fluid mixed with physiological saline solution. Smaller amounts were ineffective in this particular case. In another experiment, spinal fluid incubated with the drug in dilutions up to 1:1,000 (0.8 mg. %) for 24 hours failed to show growth; the same specimen incubated with sulfanilamide in a dilution of 1:10,000 (0.08 mg. %) showed some growth of meningococci while the control tube of spinal fluid mixed with saline solution grew out profusely. Similar experiments revealed that large numbers of meningococci present in incubated spinal fluid, when treated with sulfanilamide, may fail to grow or may grow less profusely than in spinal fluid mixed with physiological saline solution. Notwithstanding this result, no conclusion should be drawn concerning a true bactericidal action of sulfanilamide upon the meningococcus present in spinal fluids.

In summary, sulfanilamide exerts a bacteriostatic effect upon the meningococcus present in spinal fluid obtained from patients with meningococcal meningitis. Clinical observations may show whether this kind of experiment can give an indication of the effectiveness of this drug in the treatment of meningococcal meningitis.

9731 P

Experimental Street Virus Rabies in White Mice. Studies on Passive Immunization. II.*

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In a recent publication[1] from our department it was reported that white mice could be afforded a certain degree of passive protection against intracerebral inoculations of rabies street virus if large amounts of antirabic serum were injected intraäbdominally before the administration of virus. One experiment indicated that rabbit antirabic serum gave better protection than had previously been demonstrated for goat antiserum. The present report covers further evidence of the value of rabbit antirabic serum.

Rabbits were immunized by weekly intramuscular injections of 5 cc. of a 5% fixed virus suspension in saline solution. Phenolized virus (½% phenol) was used for the first few injections; fresh virus was employed thereafter and immunization continued for at least 10 weeks. The serums of several rabbits were pooled, filtered and inactivated before use.

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* This work was made possible through a grant from Mrs. John Sherman Hoyt.

[1] Hoyt, Anson, and Gurley, M. Katherine, PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 454.

Table I shows the protective value of rabbit antirabic serum when injected before intracerebral inoculations of street virus; three experiments are summarized. Two strains of street virus were employed, one from a dog, the other consisting of brain tissue from a human case of rabies. The virus was ground to a 1 to 10 suspension in saline solution and centrifuged; the supernatant fluid was inoculated intracerebrally in doses approximating 0.02 cc.

TABLE I.

Antirabic Serum Injected Intraabdominally Before Intracerebral Inoculation of Street Virus.

Source of Virus	Exp. No.	No. of Mice	Amt. Serum, cc.	Survivals	Mice Contracting Rabies	% of Survivals
Dog Brain	I	26	2	26	0	100
,, ,,	II	14	2	13	1	93
,, ,,	II	15	1	13	2	87
Human Brain	III	21	1	21	0	100
Dog Brain	II	15	½	9	6	60
,, ,,	II	14	¼	7	7	50
,, ,,	II	14	$\frac{1}{10}$	6	8	43
Controls	I	27	None	0	27	0
	II	26	,,	0	26	0
	III	31	,,	3	28	10

Exp. I. Discarded on 22nd day. Mean incubation period of controls 11.0 days.
Exp. II. ,, ,, 31st ,, ,, ,, ,, ,, ,, 11.9 ,,
Exp. III. ,, ,, 31st ,, ,, ,, ,, ,, ,, 15.3 ,,

The protective power of rabbit antirabic serum was further assayed in one experiment by the injection of the serum at varying intervals following the intracerebral introduction of virus. Human street virus, in doses already indicated, was employed and serum was administered intraäbdominally in 1 cc. amounts. All mice were observed for 31 days. Table II summarizes the results of this experiment.

TABLE II.

Injection of Serum at Varying Intervals Following Intracerebral Introduction of Virus.

Serum Given	Total Mice	Survivals	Mice Contracting Rabies	% of Survivals	Mean Incubation Period of Mice Contracting Rabies (Days)
1 day before virus	21	21	0	100	—
2 hrs. after virus	19	19	0	100	—
1 day ,, ,,	18	16	2	89	25.3
2 days ,, ,,	21	19	2	90	26.0
4 ,, ,, ,,	20	11	9	55	23.6
Controls, no serum	31	3	28	10	15.3

Under the experimental conditions employed rabbit antirabic serum injected intraperitoneally into mice showed a marked protective power against intracerebral inoculation of rabies street virus even when serum was administered as late as 4 days after virus.

9732 P

A Pannus-forming Infection of Sheep Eyes.

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From the Laboratory of Surgical Research, Division of Ophthalmology, Western Reserve University School of Medicine, and the University Hospitals, Cleveland, Ohio.

This preliminary report is concerned with the description of a disease of the conjunctiva and cornea of sheep, of natural occurrence, which results in the formation of a pannus, not unlike that in human trachoma.

The farmer from whom I obtained my diseased sheep first noticed the non-purulent infection of the conjunctiva and nictitating membrane, evidenced by congestion, edema, epiphora, as fall weather and near-freezing temperatures prevailed. Examination by him of the large flock allowed about 200 infected animals to be isolated, 10% of which had marked vascularization of the cornea. Two weeks later, when I was asked to examine the flock, less than 1% were not infected. Probably 80% suffered clinical pannus formation, and 5% superficial ulcers at the tip of the pannus. Hypopyon ulcer was seen in only those that were so blinded as to be unable to prevent injury to the cornea in trying to graze and follow the flock. No perforation of the cornea was seen. A representative animal, moderately infected, was removed to the laboratory for research purposes.

Animals in the locality had, during the summer, been infected with equine encephalitis, gangrenous stomatitis (calf diphtheria, necrobacillus, or actinomyses necrophous-Bergey's classification) and contagious ecthyma. Adjoining farms enjoying identically good pasture and climatic conditions, suffered the above 3 infections, but none had sheep with infected eyes. An Indian boy who had attended the sheep during lambing was examined, but showed no evidence of clinical trachoma.

A number of organisms have been cultivated from conjunctival scrapings; these have been common saprophytic bacteria. In smears of material removed by scraping, and stained by Giemsa's method, many epithelial cells can be seen to contain structures identical, morphologically, with *Rickettsia conjunctivæ*, as described by Coles.[1] The disease can be transmitted from sheep to sheep but we have not, so far, been able to cause infection in other animals. In sheep, definite evidence of infection is present in 48 hours after inoculation, the conjunctiva resembling that of inclusion blenorrhea. Experimental transmission proceeds more rapidly when the room temperature is below 60°. Follicles are not a prominent feature and are confined to the fornix. Within 4 days, large arteries with "rocket-like" tips are invading the *substantia propria* near Bowman's membrane, more advanced from above than below but, to a degree, completely around the limbus. The arteries appear more superficial than the veins when the circulation is studied with slit-lamp and corneal microscope. Beyond the largest, most rapidly advancing vessel tips are often seen small accumulations of cells that give the appearance of having advanced ahead of the endothelial lining. In none of the experimental infections has the pannus extended beyond the limit of the pupil; of those occurring naturally in the field, many showed eyes in which the pannus from above and below intersected at the center of the cornea. Pathological examination of one such eye showed some interstitial vascularization. In moderately vascularized corneæ, the vessels are confined to the superficial stroma. Slit-lamp examination during the early stages of rapidly progressing pannus suggest that the vessels start in an arrangement similar to the spacing of the corneal nerves. This disease offers an excellent opportunity for the study of pannus formation.

The description of the infectious agent, extensive remarks concerning observations made with the slit-lamp, and pathological findings will be published at a later date.

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[1] Coles, J. D. W. A., 17th Report of the Director of Veterinary Services, Onderspoort, Pretoria, South Africa, 1931, Sec. II, 175.

9733

A Study of Visceral Pain as Related to the Biliary Tract.

George S. Bergh and John A. Layne. (Introduced by O. H. Wangensteen.)

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Because of the prevalence of disease of the gall bladder and bile ducts, the nature of biliary colic is of extraordinary interest. Recently we have had an opportunity to conduct studies of biliary tract pain upon unanesthetized human subjects, and our findings form the basis for this report.

Patients who had undergone choledochotomy and intubation of the common bile duct several weeks or months previously voluntarily submitted themselves as subjects for these studies. As a preliminary step the condition of the biliary tract was determined by cholangiographic examination. Pain was then produced by sudden distention of the ducts by the introduction of sterile physiological saline solution under pressures varying from 50 to 100 cm. of water. This was accomplished by attaching an infusion flask containing saline solution to the T-tube leading into the common bile duct, and adjusting the pressure by elevating or lowering the flask. The patients were then observed closely to determine the effects of the procedure. Although they were interrogated concerning their sensations during the period of the experiments, leading questions were carefully avoided. In fact, much of the information was volunteered by the subjects without the necessity for questioning.

Among the 15 patients studied, all suffered deep epigastric or right upper quadrant pain following sudden distention of the bile ducts. In 8 of the cases the sensation remained localized while in the remaining 7 there was radiation to the interscapular or right subscapular region. The deep epigastric or right upper quadrant pain occurred earlier than the referred pain by many seconds. In most cases the pain was intense, but occasionally it consisted of only a relatively mild discomfort.

Gradual distention to a pressure of 100 cm. of water, on the other hand, failed to produce pain.

One might expect that those patients experiencing reference of pain to the back prior to operation would suffer a similar subscapular or interscapular pain when the ducts were distended, but no such correlation could be made in our series. The patients, however, without exception stated that the pain in the epigastrium or right upper quadrant was practically identical with the pain previously experienced from their disease.

TABLE I.
Tabulation of Results of Distention of the Biliary Ducts.

Case	Pressure (cm. of water)	Pain in epigastrium and right hypochondrium	Radiation of pain to the back		Nausea and vomiting	Eructation
			Pre-operative	Upon distention of bile ducts*		
1	60	present	present	present	absent	absent
2	52	"	"	absent	"	"
3	51	"	"	"	present	"
4	55	"	"	present	nausea only	"
5	100	"	absent	"	absent	present
6	100	"	present	absent	"	absent
7	†	"	"	"	"	"
8	60	"	absent	"	"	"
9	†	"	present	present	nausea only	"
10	50	"	"	absent	absent	"
11	50	"	"	present	"	present
12	60	"	"	"	"	absent
13	50	"	"	absent	"	"
14	50	"	absent	"	"	"
15	55	"	present	present	"	"

*The pain in the back developed later than the deep epigastric and right upper quadrant pain.
†Not recorded.

Associated with the pain there was a marked rigidity of the muscles of the abdominal wall, greatest in the right upper quadrant. Inspiratory distress was a frequent accompaniment. It was impossible to examine for localized tenderness because of the intensity of the pain already present.

There was vomiting in only one case, and one other patient vomited a short time after the completion of the experiment. A feeling of fullness in the epigastrium was frequently experienced and 2 patients belched when the ducts were distended. One patient fainted, presumably as a result of the severity of the pain when the biliary ducts were distended.

Our observations are interesting inasmuch as painful sensations identical with biliary colic were produced by distention of the bile ducts. Our results differ from those of Zollinger,[1] who failed to produce reference of pain to the back by distention, in that subscapular of interscapular pain occurred in 7 of our 15 patients. It is significant, moreover, that in all of our cases the deep epigastric or right upper quadrant pain preceded the development of the pain in the back by an appreciable period of time.

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[1] Zollinger, Robert, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1260.

9734 P

Effects of Intravenous Injections of Acacia on Certain Functions of the Liver.

W. Knowlton Hall. (Introduced by J. A. Greene.)

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The investigations of Andersch and Gibson[1] as to the fate of intravenously injected acacia, showed that a large proportion of the acacia was deposited in the hepatic cells of the liver, rather than being excreted as had been previously assumed by many writers. In view of the extensive clinical use of acacia in shock, hemorrhage, nephrosis and other conditions, it seemed advisable to make a more extensive study of the effects of the deposited acacia upon various functions of the liver.

The importance of the liver in carbohydrate metabolism leads one to expect significant changes in tolerance to carbohydrates if that organ were damaged by the deposition of the gum. It is at least partially true that the extent to which a particular sugar is converted into liver and muscle glycogen probably determines the degree of hyperglycemia which results following its ingestion.[2] It was long thought that galactose was specifically metabolized by the liver.[2] However, the dog is able to metabolize considerable amounts of this sugar in the absence of the liver.[3]

Glucose and galactose tolerances were done on 5 normal dogs after they had fasted 18 hours or more. After taking blood from the marginal vein of the ear for a sugar determination,[4] 1½ gm. of the sugar per kilo of body weight were given by stomach tube. Blood for sugar determinations was taken every half hour over a 2-hour period. After the dogs had been given a number of intravenous injections of acacia (a total of 4.4-10.7 gm. per kilo) at 3-day intervals, the glucose and galactose tests were repeated.

In every case, with the 5 dogs, we found a marked diminution of tolerance to galactose, and a somewhat less marked drop in glucose tolerance after acacia injections. The reduced carbohydrate tolerance would seem to indicate impairment of the function of the liver for carbohydrate metabolism. There are many cases reported in the literature both of humans and of experimental animals which show that extensive liver damage is associated with a reduction in the tolerance to galactose,[2] though an animal with 80% of the liver removed still utilizes glucose and galactose normally.[5]

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[1] Andersch, M., and Gibson, R. B., *J. Pharm. and Exp. Therap.*, 1934, **52**, 390.

[2] Soffer, L. J., *Medicine*, 1935, **14**, 185.

[3] Deuel, H. J., *Physiol. Rev.*, 1936, **16**, 173.

[4] Gibson, R. B., Proc. Soc. Exp. Biol. and Med., 1929, **27**, 480.

The liver seems to be the principal if not the sole source of the blood plasma proteins,[6] consequently, "in advanced chronic hepatic lesions there is a moderate reduction in the total serum proteins, the diminution occurring chiefly in the albumin fraction . . . ".[6] The diagnostic and prognostic value of these changes in plasma proteins as indicative of liver damage have been repeatedly emphasized.[6, 7] Dick, Warweg and Andersch[8] have shown that such a change takes place in the plasma proteins of dogs after acacia injections, and that the drop is much greater than can be accounted for by the dilution effect of the acacia.

TABLE I.
Effect of Intravenous Injections of Gum Acacia Solution upon Tolerance of Dogs to Glucose and Galactose.

Dog	Acacia total gm./kg.	Glucose tolerance. Bl. sug. at half hrs. mg.% as glucose					Galactose tolerance. Bl. sug. at half hrs. mg.% as glucose				
		0	.5	1	1.5	2	0	.5	1	1.5	2
1	0	100	147	107		81	92	157	145	131	129
	4.4	120	157	157	140	98	98	200	236	212	162
2	0	81	118	107	82	79	81	162	165	192	173
	7.5	69	165	179	138	79	90	131	189	231	175
4	0	50	150	122	80	78	87	135	122	96	85
	9.0	80	170	147	90	95	90	142	186	157	102
5	0	92	179	173	90	74	120	167	212	150	142
	10.7	96	217	212	165	96	102	102	226	212	167
6	0						79	167	186	159	129
	8.4						86	186	226	182	138

Plasma protein determinations were done by the method of Andersch and Gibson[9] on blood drawn from 4 dogs before acacia injections were made and again several days after the last injection. In general, our results confirm the results of Dick, Warweg and Andersch.[8] The total plasma protein with 4 dogs before and after the series of acacia injections was as follows: dog 2, 4.98% and 4.54%; dog 4, 5.67% and 4.93%; dog 5, 4.70% and 2.86%, and dog 6, control lost and 4.20%.

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[5] Bollman, J. L., and Mann, F. C., *Ann. Int. Med.*, 1935, **9**, 617.

[6] Snell, A. M., *Proc. Staff Meet. Mayo Clin.*, 1935, **10**, 489.

[7] Myers, W. K., and Keefer, C. S., *Arch. Int. Med.*, 1935, **55**, 349.

[8] Dick, W. M., Warweg, E., and Andersch, M. A., *J. Am. Med. Assn.*, 1935, **105**, 654.

[9] Andersch, M., and Gibson, R. B., *J. Lab. and Clin. Med.*, 1933, **18**, 816.

It has been repeatedly demonstrated that blood fibrinogen comes from the liver.[6] The markedly delayed clotting of the blood and fragile fibrous appearance of the clot which is formed seems to indicate that the deposition of acacia in the liver markedly affects factors in the clotting mechanism which have their origin in that organ. The average fibrinogen values for the blood from 4 dogs, drawn several days after the last acacia injection was 0.16% as compared to 0.37% before injection.

The results of this investigation indicate that the deposition of acacia in the livers of dogs produces impairment of liver function in carbohydrate and plasma protein metabolism.

9735

The Tonotropic Action of Acetylcholine on the Auricle of the Terrapin, (*Pseudemys elegans*).

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Tonic oscillations of the auricular musculature in certain species of terrapins is a well established fact.[1] This tonicity apparently depends upon the presence of auricular smooth muscle[2] since tonus is not found in the auricles of species which do not contain it. Tone is increased by pituitrin, histamine, nicotine, potassium chloride and barium chloride and depressed or abolished by epinephrine and tyramine.[4, 5] Tonus is generally believed to be increased by stimulation of the vagus.[1, 6] Recently, however, it has been reported that the tonus mechanism is not innervated by parasympathetic motor nerves since it is not influenced by physostigmine or atropine.[7]

Therefore, for a more complete understanding of this mechanism, it seemed necessary to know the action of acetylcholine on tonus.

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[1] Fano and Fayod, *Arch. Ital. de Biol.*, 1888, **9**, 143.

[2] Laurens, H., *Anat. Rec.*, 1913, **7**, 273.

[3] Chu, Hung-pih, *Chinese J. Physiol.*, 1930, **4**, 263.

[4] Gruber, C. M., and Markel, C., *J. Pharm. and Exp. Therap.*, 1919, **12**, 435.

[5] Gruber, C. M., *J. Pharm. and Exp. Therap.*, 1921, **16**, 405; PROC. SOC. EXP. BIOL. AND MED., 1926-27, **24**, 92.

[6] Botazzi, P., *Arch. Ital. de Biol.*, 1900, **24**, 17.

[7] Greene, C. W., and Maneval, K. E., *J. Pharm.*, 1932, **46**, 303

In the experiments here described, the terrapin *(Pseudemys elegans)* was used. The auricles were carefully cut away from the ventricle and large veins and suspended by threads between the tip of a glass L-tube and a heart lever. This was then suspended in a muscle bath and bathed with Ringer's solution. A small stream of air was delivered to the solution by the L-tube throughout the experiment. The solutions used were at room temperature (18-20°C.). The concentrations of the solutions referred to in the text indicate the concentration of the drug in the muscle bath. In Ringer's solution, strong rhythmic contractions were obtained, these usually continuing for many hours. Only occasionally were tonus waves absent.

The addition of acetylcholine increased the tonic oscillations. The sensitivity of the auricular preparation to acetylcholine varied greatly from one experiment to another. The actual concentration used with any particular preparation is not significant. Often a definite response could be elicited by 1:50,000 and again with another preparation responses were obtained only with 1:1000. With an effective concentration of acetylcholine, the auricle responds with inhibition of the fundamental rhythm along with a sustained increase in tonus resembling a "contracture", the amplitude of which was often greater than that of the fundamental rhythm (Fig. 1).

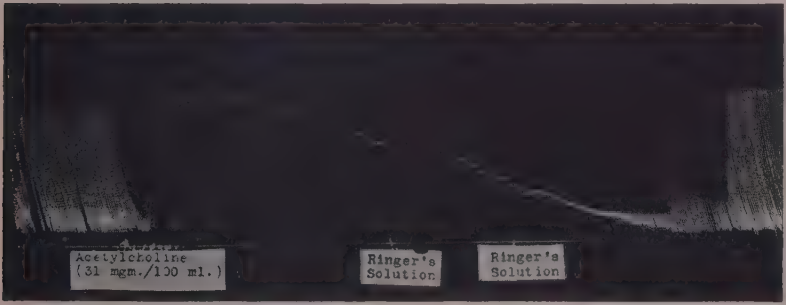

FIG. 1.
Acetylcholine to make a concentration of 31 mg. per 100 ml. of solution induces a strong tonic contraction of the auricle.

This action of acetylcholine is prevented or abolished by atropine, (1:20,000).

Acetyl beta methylcholine chloride has the same action as acetylcholine, often inducing an increase in tonus in dilutions of 1:200,000. Atropine prevents or abolishes this response.

The addition of a potassium excess in Ringer's solution or the substitution of a 0.7% solution of potassium chloride induced tonus responses almost identical to those described for acetylcholine. They are usually of longer duration and are not prevented by the previous administration of atropine. Epinephrine inhibits the tonus response whether the stimulating agent is acetylcholine or potassium chloride (Fig. 2). In agreement with Gruber[8] pilocarpine hydrochloride was found to exert a markedly depressant action on the fundamental rhythm of the auricles but had no influence on the tonus waves.

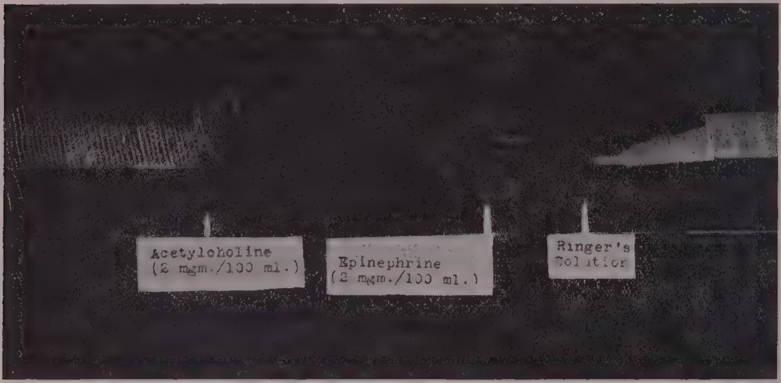

FIG. 2a.

Acetylcholine to make concentration of 2 mg. per 100 ml. of solution induces a strong tonic contraction. Epinephrine shortens the recovery period.

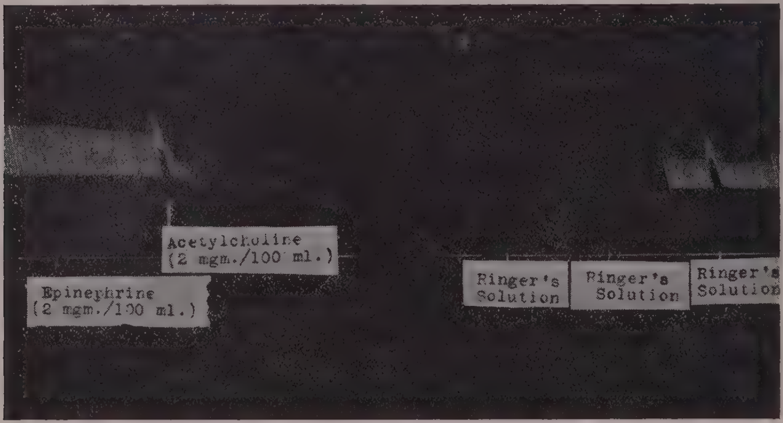

FIG. 2b.

The same preparation as in Fig. 2a, but epinephrine to make 2 mg. per 100 ml. of solution prevents a response to acetylcholine.

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[8] Gruber, C. M., *J. Pharm. and Exp. Therap.*, 1920, **15**, 23.

9736

Hyperglycemic Response to Hypoglycemia in Diabetic and in Healthy Individuals.

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We described[1] experiments on dogs which show the frequent occurrence of hyperglycemia as the aftermath of hypoglycemia in the postabsorptive state. We had previously made the same observation on diabetic and nondiabetic human subjects. The phenomenon attained particular interest because it explains the familiar extreme fluctuations of the blood sugar level in insulin-treated severe diabetics that perplexed investigators since the advent of insulin. An understanding of the nature of these fluctuations made possible a satisfactory clinical management of such cases.

The extent of the compensatory hyperglycemia in nondiabetic subjects is moderate so that its existence assumed significance only in the light of our observations on diabetic patients. The phenomenon was found to occur in numerous nondiabetic individuals in the course of the conventional glucose tolerance test. In the majority of healthy persons, as is known, the hyperglycemia that obtains for a period of about one hour or 90 minutes after the ingestion of 100 gm. of glucose, recedes close to the postabsorptive (fasting) level at the end of about 2 hours. An hour later, *i. e.*, 3 hours after the intake of sugar, a hypoglycemic dip (5 to 20 mg. %) below the fasting level frequently occurs, and in another hour the fasting level is restored.

Individual variations and deviations from this course, however, are frequent. In many instances directly after the hypoglycemic phase a secondary hyperglycemia appears before the fasting level is reëstablished. It is this phenomenon with which we are concerned. To illustrate it, we present the results of 23 glucose tolerance tests performed on nondiabetic individuals. The first 14 of these cases exhibit distinct secondary hyperglycemic levels at the end of 3 hours after the ingestion of glucose, the rise above the fasting values ranging from 8 to 29 mg. %. This hyperglycemia is preceded by a hypoglycemic drop at the end of the second hour. In the remaining 9 cases given in Table I, marked hyperglycemia was observed at the end of the fourth hour; in a few of these cases hyperglycemia was present at the end of the third hour but increased further during the fourth. The greatest rise in this group was 33 mg. % above the fasting level.

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[1] Somogyi, M., Weichselbaum, T. E., and Heinbecker, P., PROC. SOC. EXP. BIOL. AND MED., 1937, **32**, 65.

TABLE I.
Response to Glucose Tolerance Tests in Nondiabetic Subjects Showing Secondary Hyperglycemia.

No. of case	Time after ingestion of 100 gm. of glucose (hours)					Secondary rise above fasting level
	0	1	2	3	4	
	Mg. true sugar per 100 cc. of blood.					
1	88	90	60	96	74	8
2	70	80	64	79	—	9
3	92	139	70	103	—	11
4	91	158	83	103	102	12
5	86	90	84	98	—	12
6	93	138	88	109	84	13
7	77	97	82	93	—	16
8	76	97	57	93	73	17
9	67	91	65	85	83, 57*	18
10	82	142	83	101	81	19
11	82	122	80	101	—	19
12	86	132	72	109	89	23
13	80	110	84	105	81	25
14	83	176	98	112	92	29
15	93	104	74	88	98	5
16	79	119	107	81	89	10
17	90	133	93	96	101	11
18	73	107	76	82	85	12
19	80	136	89	83	93	13
20	90	116	112	78	105	15
21	81	138	124	80	99	18
22	86	149	131	97	105	19
23	82	108	93	87	115	33

*Five hours after the ingestion of glucose.

It may be noted that in some of the cases no actual hypoglycemia is recorded, that is to say, the blood sugar dropped only to or near the fasting level, yet a hyperglycemic after-effect ensued. A substantial and relatively rapid drop of the blood sugar from hyperglycemic levels apparently sufficed in these instances to elicit a subsequent rise, without the necessity of attaining a true hypoglycemia. It is likely also that in some instances the hypoglycemic stage has been missed since blood sugar determinations were carried out only at hourly intervals.

That hyperglycemia is in general a physiologic response to hypoglycemia, is indicated by the fact that it occurs independently of the causes that are responsible for the hypoglycemia. Thus in experiments on dogs hyperglycemia was observed as a response to hypoglycemia in the course of glucose tolerance tests, and also as the aftermath of hypoglycemia that was produced by the injection of small doses of insulin.[1] Again entirely different experimental conditions are at work in bringing about the so-called "arbeitshyperglykemie." This phenomenon occurs during strenuous physical exertion performed in the postabsorptive state. A rapid initial drop of the blood sugar under these conditions is followed by a rise above the fasting level. Similarly in artificial fever, with rising body temperature, hypoglycemia frequently sets in and is promptly followed by hyperglycemia (unpublished observations made in this laboratory).

The blood sugar level, therefore, seems to be the primary factor that influences the equilibrium of the reversible enzymatic reaction, glycogen ⇌ blood sugar, in the liver. One must postulate on this basis that hypoglycemia, in order to produce a hyperglycemic aftereffect, causes a shift in the balance of the reaction by increasing its velocity in the direction of glycogen breakdown (to the right) relative to the reaction velocity in the direction of glycogen formation. (Hyperglycemia causes a reversal of this relationship.)

The accommodating capacity of the enzymatic reaction, *i. e.*, its elasticity and quickness of response to changing blood sugar levels, exhibits considerable individual variations. In many normal individuals and experimental animals the glycogen breakdown, when stimulated by hypoglycemia, proceeds at an increased rate only until the normal postabsorptive sugar level had been restored, the process slowing down in time to avoid an overstepping of this limit. This course represents the regulatory system at its best, in that it provides maximum stability in the readjustment of the blood sugar level. In other healthy subjects and animals, however, the enzymatic glycogen breakdown, once stimulated, maintains an accelerated pace for some time after it has satisfied the demand, producing a transitory hyperglycemia of moderate degree. The cases in Table I belong to this group. We interpret this as an expression of a slight degree of inefficiency of the regulatory system, conducive to a mild form of instability in the readjustments of the blood sugar level. A more pronounced instability is exhibited in a third group, where hyperglycemic and hypoglycemic phases alternate repeatedly before the final readjustment takes place. Examples of this condition are cases No. 1, 6, and 9 in Table I and the 3 cases in Table II.

We have found that these differences in the response to glucose tolerance tests do not by any means represent accidental variations that may occur in the same individual from one day to another. On the contrary, a given person responds to repeated tolerance tests with the same type of curve (provided, of course, that no essential

TABLE II.
Repetition of Glucose Tolerance Tests Shows Consistency in Type of Response.

No. of case and date of test	Time after ingestion of 100 gm. of glucose (hours)					Secondary rise above fasting level
	0	1	2	3	4	
	Mg. true sugar per 100 cc. of blood.					
1. July 7	76	97	57	93	73	17
July 10	81	134	78	92	55	11
2. Feb. 11	81	65	73	88	69	7
Apr. 11	76	70	78	86	64	10
3. Nov. 23	94	119	88	106	88	12
Nov. 26	87	105	80	111	—	24

change has taken place in his dietary regimen and physical condition). It appears, therefore, that the type of response of nondiabetic persons to glucose tolerance tests expresses characteristic individual qualities. A few examples, given in Table II, show that individuals in whom a glucose tolerance test indicated lability of the regulatory mechanism, exhibited a similar type of response when the test was repeated.

In the diabetic condition hypoglycemia exerts the same effect upon the hepatic enzyme system as in the nondiabetic, but in a greatly exaggerated degree. The difference between the processes in the normal and in the pathological condition is quantitative rather than qualitative. In diabetes (irrespective of its etiologic causes, and aside from the influence of the muscle tissues upon the blood sugar level) the prevalence of hyperglycemia attests to a dislocation in the balance between the 2 opposite enzymatic processes in the liver, in the sense that the rate of glycogen breakdown materially outstrips the rate of glycogen formation. If in addition the stimulating effect of hypoglycemia is superimposed upon such an unbalanced enzyme system, it is understandable that the ensuing compensatory swings will be far greater than in the nondiabetic, resulting in excessive hyperglycemia and glycosuria.

Our experience has convinced us that the wide fluctuations in the blood sugar and the progressively increasing instability of diabetic patients are the direct results of the administration of excessive doses of insulin over a long period of time. (In this relation we define as an excessive dose any amount of insulin that causes hypoglycemia.) In our experience adequate reduction of the insulin dosage in conjunction with a liberal dietary regimen eventually restores such patients to a greatly improved stability. This will be shown in forthcoming clinical reports.

Summary. 1. In numerous healthy individuals and normal experimental animals hypoglycemia entails hyperglycemia in the postabsorptive state, irrespective of the experimental conditions which initiated the hypoglycemia. The phenomenon is interpreted as the result of a delay in the adjustment between the reaction velocities of glycogen breakdown and glycogen formation in the liver. 2. In the diabetic the same physiologic process takes place on a greatly magnified scale. Hypoglycemias, caused by overdoses of insulin, entail in the diabetic patient excessive degrees of hyperglycemia and glycosuria. Recurrence of this sequel over considerable periods of time progressively increases the instability of the patient and aggravates the disease.

9737

Vitamin C Saturation—Kidney Retention after an Intravenous Test Dose of Ascorbic Acid.

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Following the observation that Vitamin C was not uniformly absorbed from the intestinal tract it was natural that the intravenous route of administration should be adopted for the test dose* and for therapy when utilization by the oral route was unsatisfactory. This method of studying the Vitamin C saturation of the body was described by the authors,[1, 2] using 1000 mg. doses and later by others[3, 4] with smaller dosage. When the 1000 mg. test dose in 10 cc. of physiologic solution of sodium chloride was given intravenously the normal urinary excretion during the 24 hours following was found to be not less than 500 mg. In deficient states the body uses more of the vitamin and the excretion is less. In saturated states the excretion may be markedly increased. Fever and the use of certain drugs may influence metabolism and excretion. At whatever level studied it was found that 80% or more of the amount excreted in the 24 hours was excreted in the first 5 hours.[2] Exceptions to this were very rare and we have never seen a reading below 75%. This was the basis of the 5-hour test after an intravenous test dose, the normal excretion, therefore, being not less than 400 mg. More than 200 such tests have confirmed our original studies and the conclusion that this represented the most satisfactory test for Vitamin C saturation suggested up until that time. The advantages of 5-hour studies over 24-hour studies are obvious if equally accurate. Single observations of the blood content of Vitamin C are too dependent upon intake or lack of intake of the vitamin during the preceding 48 hours. Urinary excretion studies without a test dose had proven very unreliable and the smaller doses for the intravenous method appear to us to be too markedly influenced by the immediately preceding diet.

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* The ascorbic acid used was supplied through the courtesy of Merck and Company, Rahway, N. J.

[1] Wright, Irving S., *Am. J. Med. Sci.*, 1936, **192**, 719.

[2] Wright, Irving S., Lilienfeld, Alfred, and MacLenathen, Elizabeth, *Arch. Int. Med.*, 1937, **60**, 264.

[3] Ralli, E. P., Friedman, G. J., and Kaslon, M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 52.

[4] Finkle, Philip, *J. Clin. Invest.*, 1937, **16**, 587.

There was, however, a weakness in this and all similar tests which we pointed out.[2] Having overcome the factor of faulty absorption from the gastro-intestinal tract we were still confronted with the possible factor of faulty elimination through the kidneys. We suggested that a parallel blood curve determined during the 5-hour test would probably enable us to derive the information regarding kidney excretion and provide a more completely satisfactory saturation test. We have, therefore, proceeded with such studies as follows:

The patient was permitted his normal diet with no increase in citrus fruits (since the amount of Vitamin C taken in the ordinary diet in 24 hours does not appreciably alter the findings when 1000 mg. doses are used) but fasted on the morning of the test. All possible urine was voided and discarded. Control specimens of blood were taken and 1000 mg. of ascorbic acid in 10 cc. of physiologic saline was injected intravenously. Specimens of blood and urine were collected and analyzed for Vitamin C content at 15 minutes, 1½ hours, 3 hours and 5 hours after the injection respectively.†

We had previously reported[2] typical blood curves following such a procedure with individuals in varying states of Vitamin C sat-

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† The vitamin C in the urine was determined by a modification of Tillman's 2.6-dichloraphenolindophenol method[5] and in the blood by the method of Farmer and Abt.[6]

[5] Tillman, J., Hirsch, P., and Jackisch, J., *Z. f. Untersuch d. Libersmith,* 1932, **63**, 241.

[6] Farmer, C. J., and Abt, A., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1625.

TABLE I.

Intravenous 1-gram Test for Vitamin C Saturation. Typical Blood and Urine Figures in Patients with and without Renal Disease.

	History	Control Blood mg. %	15 min.		1½ hr.		3 hr.		5 hr.		Total Urine mg. C
			Blood mg. %	Urine mg.	Blood mg. %	Urine mg.	Blood mg.%	Urine mg.	Blood mg.%	Urine mg.	
M.A.	C intake excellent. No renal disease	1.2	8.6	64.5	4.4	440.3	3.0	189.7	2.4	75.2	789.7
H.R.	C intake poor. No renal disease	.33	3.3	10.0	1.3	112.7	.86	78.0		1.8	131.3
C.D.	C intake good, Glom. neph. No nitro. retention	.88	4.7	110.4	2.4	273.0	1.6	89.6	1.4	51.1	524.1
M.T.	C intake excellent. Malig. Hyper. N.P.N. 75 mg.	1.5	4.6	2.1	4.1	26.3	4.1	23.7	2.2	26.0	78.1
F.D.	C intake poor. Glom. neph. N.P.N. 53 mg.	Broke in centrifuge	6.1	6.8	2.6	19.4	1.6	10.8	1.4	5.3	42.3

uration but with no evidence of retention at the kidney threshold. These curves were confirmed and we then performed the same tests in patients with several types of renal disease to see whether retention of this vitamin occurred as a result of certain types of kidney damage. In cases illustrating some of the possible types of results the findings were as shown in Table I.

The curves of patients M. A. and H. R. were selected as being typical of those seen in individuals without renal retention of Vitamin C. The 15-minute blood reading is always the highest and there is a fairly sharp drop with the blood level returning well toward the control level within 5 hours. The general form of the curve is similar whether the patient is unsaturated or saturated with Vitamin C, the main difference being in the height of rise. The urinary excretion curve is also similar in form regardless of degree of saturation of the individual in that the peak is reached in 1½ hour specimen followed by a rapid return toward the control level. Again the difference rests in the height of the rise.

Patient C. D., a typical case of glomerulonephritis, had a good dietary history for Vitamin C. There was no nitrogenous retention. Both the blood and urinary curves for Vitamin C were typical for individuals with normal saturation and no renal retention of Vitamin C.

Patient M. T. was suffering from advanced malignant hypertension with kidney damage and nitrogenous retention in the blood (N.P.N. 75 mg. per 100 cc. One week later 125 mg. per 100 cc.). In spite of the preceding excellent dietary history for Vitamin C the low urinary excretion would lead one to believe that the body was very low in the vitamin unless a blood curve were also followed. The blood figures show a retention of the Vitamin C in the blood stream probably because of faulty renal excretion. This avoids an error of interpretation inevitable with the urinary excretion studies alone.

Patient F. D. was suffering from glomerulonephritis with nitrogenous retention in the blood. (N.P.N. 53 mg. per 100 cc. One week later 75 mg. per 100 cc.) The dietary history for Vitamin C was only fair. In this patient the blood curve would appear to be normal in form without renal retention but the urinary curve demonstrates that this was not due to excretion through the kidneys. The most likely explanation rests in the probability of very rapid utilization by the tissues due to their previous dietary depletion.

Thus far we have obtained evidence of Vitamin C renal retention in cases with nitrogenous retention only, but not in all such patients.

Further studies may show that this phenomenon may occur under other conditions also.

Conclusions. 1. The intravenous test dose method for the determination of Vitamin C saturation in the body by the study of the urinary excretion eliminates the factor of uncertain absorption of the ascorbic acid from the gastro-intestinal tract, but the problem of faulty kidney elimination remains. 2. In order to properly evaluate this factor a study of the blood curve for possible renal retention is essential. 3. A test is outlined which permits a more complete study of Vitamin C saturation. 4. Results illustrating certain types of curves obtained with and without renal retention are presented and interpreted. Thus far retention of Vitamin C has been noted in patients with marked nitrogenous retention only. 5. All patients with nitrogenous retention do not have Vitamin C retention, but renal retention of Vitamin C may occur. To our knowledge this has not been demonstrated before.

9738

Sexual Abnormalities in an Inbred Strain of Mice.

ELIZABETH FEKETE. (Introduced by C. C. Little.)
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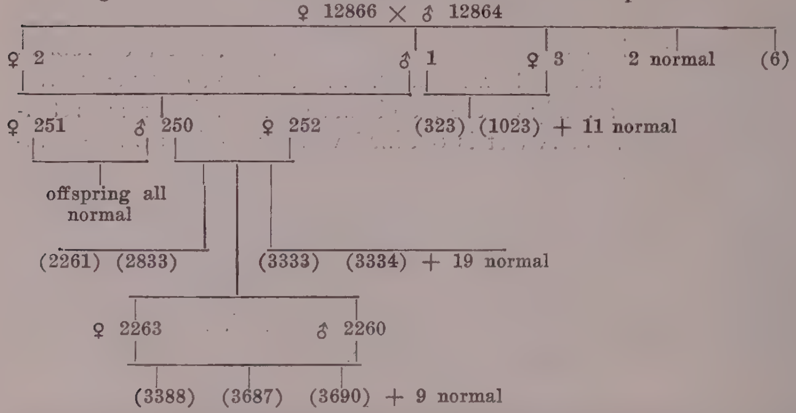
An interesting sexual abnormality was observed in the Little-Murray dilute brown strain of mice. To date this abnormality has been observed in 10 animals, and is evidently hereditary. All the abnormal individuals were descendants of the same pair of mice, the ancestors of which were inbred by brother to sister matings for many generations. A pedigree chart (Table I) gives further details.

Mouse No. 6 was 23 months old when killed. Externally the animal was a normal female. Internally no female sex organs were present. A large nodule was found in the peritoneal cavity. Microscopical sections showed that the nodule was surrounded by a fibrous capsule. A few definitely recognizable but atrophic seminiferous tubules were present near the periphery. The rest of the tissue was composed of diffusely distributed large cells, of embryonal type, and was diagnosed as embryonal carcinoma of the testis (Fig. 1).

Mouse No. 323 was 20 months old when killed. At autopsy a large, yellow, necrotic nodule was found on the right side, half way between the kidney and the bladder. On the left side a small oval body was found in the same position. Inserting a probe into the vagina, a blind ending could be felt. No other sex organs could be

TABLE I.

Pedigree Chart. The abnormal animals are marked with parentheses.



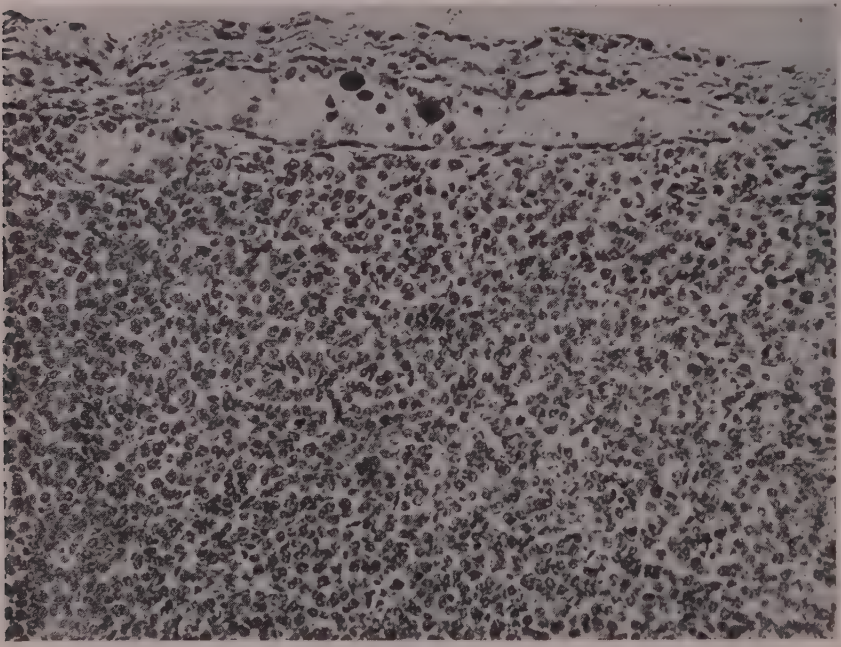


FIG. 1.

Diffuse embryonal cell carcinoma of testis of mouse No. 6 showing tunica albuginea, seminiferous tubules and some dark staining concretions. × 250.

found. Examined microscopically the yellow nodule of the right side showed almost complete necrosis. Only a very small peripheral area was still cellular, and showed faint outlines of seminiferous tubules. The small body of the left side was surrounded by a fibrous capsule and contained seminiferous tubules in which actively dividing spermatogonia and spermatocytes were present, but there were no spermatozoa. Interstitial cells were present in the stroma.

The condition found in 6 other animals was very similar. In one of them, mouse No. 3388, the position of the internal organs was sketched and serial sections were cut of all the parts that might possibly contain any accessory sex organs. The observations made on this animal will be described in more detail.

The serial sections showed that the vagina was about 5 mm. long and ended blindly. Two lobules of fat forming a pad were attached at the base of the bladder. The testes were partially imbedded in the adipose tissue half way between the kidneys and the bladder and supplied with blood vessels from the fat lobules. The whole fat pad including the testis was serially sectioned. The gonadal tissue was comparable structurally to an undescended testis. It was surrounded by a thin tunica albuginea. The seminiferous tubules were separated from each other by a fibrous stroma which at some places, especially near the periphery, contained interstitial cells. In some of the tubules the seminiferous epithelium showed degenerative changes; in others it was actively dividing. Spermatogonia and spermatocytes were present—no spermatozoa were found (Fig. 2). The sections of the surrounding fat pad showed no other sex organs.

As was stated previously, the condition found in the 5 other animals was similar to the one just described in detail. Slight variations were observed in the structure of the stroma of the testes. In 2 of the mice (3333 and 3334) the testes were quite large and contained adipose tissue, into which the seminiferous tubules were imbedded. Few interstitial cells were found near the hilum in these glands. In other animals the seminiferous tubules showed degenerative changes and complete hyalinization. Concretions were found in several testes. The sac-like ending of the vagina was similar in all of them.

It was observed that the vagina of mouse No. 3687 and 3690 also ended blindly. These animals were anesthetized and the peritoneal cavity examined. Testes were found in both of them. In 3687 the testes were left in while in 3690 they were taken out, and two ovaries of a dilute brown female were transplanted in the same position.

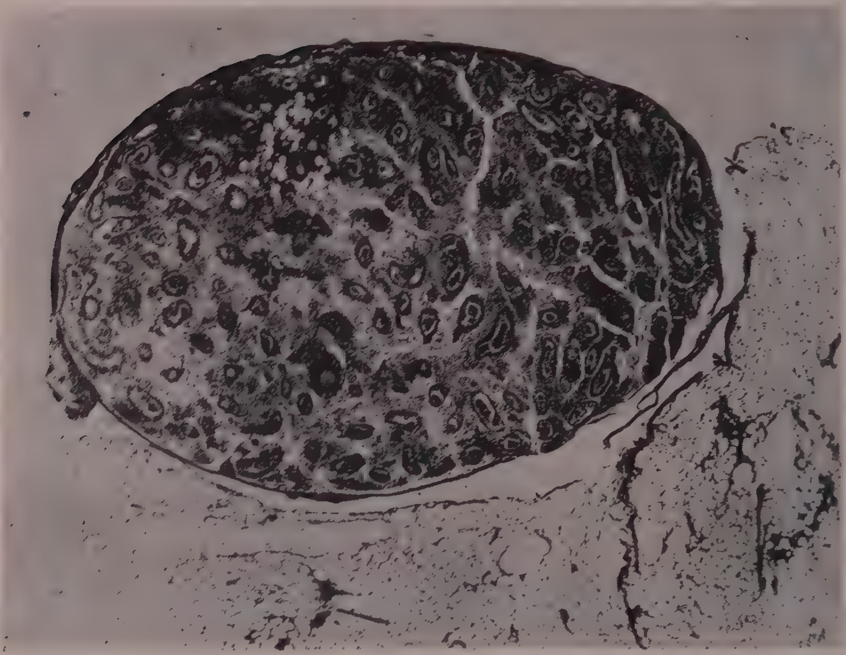


FIG. 2.
Testis of mouse No. 3388. × 25.

Mouse No. 3687 died 2 months later at the age of 26 months. The internal organs showed very marked postmortem changes. One of the testes was much enlarged and several round nodules were present on the liver. Although microscopic sections showed far advanced necrosis and definite diagnosis was impossible, it is probable that this mouse too had an embryonal cell carcinoma of the testis which gave rise to metastases in the liver. Mouse No. 3690 is still alive.

Several descendants of this line are alive and are kept under careful observation. So far no other abnormal individuals have been found.

9739

A Pressure Device for the Separation of Mammalian Spermatozoa from the Isolated Epididymis.

E. J. Czarnetzky and Werner Henle. (Introduced by Stuart Mudd.)

From the Department of Bacteriology, University of Pennsylvania School of Medicine.

During an investigation of the specificity of mammalian spermatozoa[1] it was desired to obtain a pure suspension of spermatozoa for purposes of chemical fractionation. As a very large quantity of material was needed, it was necessary to devise a means of separating them from the isolated epididymis with a minimum of labor. The following method, using a pressure device, has been found useful in rapidly separating the spermatozoa from a large number of the organs.

The pressure device consists of a foot-pedal which activates a pressure bulb fitted with 2 one-way valves in line, a water reservoir, and pressure tubing leading to a blunt No. 22 gauge hypodermic needle fitted with a shut-off valve.

The testes of bulls and rams were obtained from the abbatoir with all their coverings intact. After washing off all adherent blood clots, the cavity of the tunica vaginalis was opened and the cauda epididymis removed together with a long section of the vas deferens. These pieces of epididymis with the adherent vas deferens were placed in sterile saline until used.

The open end of the vas deferens was pulled over the blunt needle, and secured by means of a clamp, the jaws of which were covered with fine rubber tubing, to prevent cutting of the tissues and backflow. Pressure was then applied by means of the foot-pedal and water was injected into the epididymis. After injecting a small amount of water, the canal protruded in certain fields, one field appearing after the other over the shiny surface of the epididymis. When the distention became great enough to threaten rupture of the canal, an incision was made in the last field which filled up, more pressure was applied, and the spermatozoa were forced through the incision into a sterile vessel. The suspension of spermatozoa was then washed once with water. All of these manipulations were carried out with precautions to minimize contamination.*

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[1] Henle, Werner, *J. Immunol.*, 1938.

* The technic used in obtaining spermatozoa from the epididymis of smaller animals such as guinea pigs, rats, and mice has been fully described elsewhere.[1]

This mechanical device might also be found useful in the exsanguination of organs.

9740 P

Crystalline Vitamin B-6.*

JOHN C. KERESZTESY AND JOSEPH R. STEVENS. (Introduced by Hans Molitor.)

From the Research Laboratories, Merck & Co., Inc., Rahway, New Jersey.

Vitamin B-6 was designated by György[1, 2] as the "rat pellagra-preventing" principle present, as defined by his biological test, in such materials as yeast, liver, rice bran, etc. He indicated that this active substance had the properties of a nitrogen base.[3]

Starting with rice bran, we have been able to isolate the crystalline hydrochloride of a nitrogen base having the properties of Vitamin B-6 as defined by György. The hydrochloride is freely soluble in water and sparingly in alcohol and acetone. It is obtained as white platelets melting at 204-206°C. with decomposition. The chemical composition is being studied and will be reported at a later date.

The biological testing of our crystalline substance was carried out by Dr. W. L. Sampson of the Merck Institute of Therapeutic Research. The method used was the curative rat method as suggested by Moll.[4] In this procedure rats are maintained on a Vitamin B-6 deficient ration, similar to that used by György, except that a good grade of corn starch is substituted for rice starch. When the rats have developed severe symptoms of the deficiency, a single dose of the test material is administered by mouth. The effective dose is that amount which will produce a healing of the symptoms within a period of 14 days. Our crystalline preparation was tested by this method at dose levels of 0.100 mg., 0.050 mg., and 0.025 mg. The protocols are shown in Table I.

The degree of the symptoms is indicated by a scoring system similar to that given by Halliday and Evans.[5]

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* Received for publication December 30, 1937.

[1] György, P., *Nature*, 1934, **133**, 498.

[2] Birch, T. W., György, P., Harris, L. J., *Biochem. J.*, 1935, **29**, 2830.

[3] Birch, T. W., György, P., *Biochem. J.*, 1936, **30**, 304.

[4] Private communication.

[5] Halliday, N., and Evans, H. M., *J. Nutrition*, 1937, **13**, 657.

TABLE I.

	Start		After 14 days	
Rat No.	Symptoms	Weight	Symptoms	Weight
0.100 mg. level				
84-45	++++	51	0	77
86-19	++++	59	+	82
87-12	+++	39	0	59
87-31	+++	66	0	83
87-50	+++	57	0	80
89-25	+++	49	+	60
89-40	++++	58	0	84
81-6	+++	54	0	68
81-20	+++	49	+	70
89-20	++++	40	++	53
0.050 mg. level				
90-28	+++	38	+	43
84-11	+++	76	0	85
0.025 mg. level				
99-3	++	36	+++	36
99-47	++	52	dead in 12 days	
99-20	++	42	" " 11 "	

From these results it appears that a single curative dose of the crystalline preparation is no more than 0.100 mg.

9741 P

Reversed Iontophoresis of Histamine from Human Skin. Its Bearing on Histamine Theory of Allergic Wheal.

H. A. Abramson, M. Engel, V. Lubkin and I. Ochs.

From the Medical Service of Dr. George Baehr and the Laboratories of the Mount Sinai Hospital, New York City, and the Biological Laboratory, Cold Spring Harbor.

By standardization of both metallic and absorbent electrode material, an iontophoretic method has been developed which permits the detection of histamine in dilutions as high as 1:5,000,000 by the formation of wheals in the human skin.[1] The method has been serviceable in the development of a procedure for assaying histamine in human, rabbit, and guinea pig blood. Thus, histamine can be assayed directly in the blood of rabbits killed by the intravenous administration of histamine. Only 0.1 cc. of blood is required.

When histamine is administered by the galvanic current so that a wheal forms in the human skin, the positive pole is applied to force the positively charged histamine ions into the skin. It has now been found that if the negative pole be applied to the surface of the wheal formed by the scratch or by the iontophoretic method, sufficient histamine is transported out by this reversal of iontophoresis to form secondary wheals in new areas of the skin. In this way, histamine has been recovered from wheals which have been produced by iontophoresis from original dilutions as high as 1:100,000. Indeed, histamine may be recovered by diffusion alone, sufficient histamine being readily obtained for detection by means of the preceding methods within a diffusion period as short as 3 minutes. Histamine has been recovered from wheals produced by higher concentrations for as long as 40 minutes after wheal formation.

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[1] Abramson, H. A., and Alley, A., *Arch. Phys. Ther., X-Ray, Radium*, 1937, **7**, 327.

Using this method of reversed iontophoresis of histamine, attempts have been made to obtain histamine from (1) ragweed wheals, (2) timothy wheals, (3) wheals produced by ultraviolet light in a case of *urticaria solare,* (4) and wheals produced by stroking in a severe case of dermographism. It should be noted that the size of the wheals in all of these instances corresponded as well as could be ascertained by present methods with the size of histamine wheal investigated by reversed iontophoresis. In no instances (in the 4 types of wheals just enumerated) was histamine obtainable by reversed iontophoresis. Since the size and character of the allergic wheals from which histamine was not obtained corresponded in general with the size and character of the histamine wheals from which histamine was readily obtained, it seems unlikely that histamine was present in sufficient quantity in the allergic wheals to produce the whealing observed. These experiments, then, do not support the point of view that histamine or a readily diffusible substance of low molecular weight (H-substance) like histamine is responsible for the production of the allergic wheal.

9742

Extra-Hormonal Factors in Maternal Behavior.

CHARLES PHILIPPE LEBLOND. (Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University.

In rats and mice the main manifestations of maternal behavior are retrieving, licking, and cuddling of the young. Along with these activities, but often independent of them, may occur nest-building, defense reactions, and prolonged station on the nest. Nest-building, especially, is largely determined by body temperature[1] and other physiological factors[2] which do not seem to affect the principal forms of maternal behavior.

The retrieving of young is the type of maternal behavior which lends itself most readily to objective measurement. It is different from mere retrieving of material to the nest since the latter is not accompanied by licking and cuddling. It is elicited in a mouse not only by its own young, but apparently to the same degree by any young of the species which are of the same age.

The maternal behavior of the mouse was examined in 5-minute test periods with newborn young by a method previously described.[3] Only animals that had retrieved newborn in 2 test periods are reported as being maternal. Both normal and experimental animals were tested, the normals comprising virgin females, females during and after lactation, and males under 120 days of age; the experimental animals consisted of males castrated either at birth by Pfeiffer's method[4] or after puberty, males grafted with ovaries, and males injected with oestrin from birth on. The latter animals are comparable to hypophysectomized animals, at least as far as the gonads are concerned.

The results (Table I) show that maternal behavior may be elicited in almost all adult mice, whether male or female. A fairly complete pattern of behavior may be obtained in such animals. Our earlier statement[3] that the expression of maternal behavior is much less marked in non-parturient than in parturient animals is thus revised on the basis of a greatly increased number of observations. However, in males and virgin females it is usually necessary to leave newborn young in the cage of the tested animals for 1 to 4 days previous to the test. The "sensitization" of maternal behavior thus provided affected the results of the tests markedly, and is reported in Table I under the heading: "sensitization with young."

Maternal behavior was observed most readily in females during lactation. Only a small percentage of the tests was negative. This more uniform expression of maternal behavior by lactating females may be due to the continuous presence of their own young, since, as has already been pointed out, the mere presence of young over a period of time enhances the manifestation of maternal behavior.

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[1] Kinder, E. F., *J. Exp. Zool.*, 1927, **47**, 117.

[2] Richter, C. P., *Symposia on Quantitative Biology, V*, Cold Spring Harbor, Long Island, N. Y., 1937.

[3] Leblond, C. P., and Nelson, W. O., *Am. J. Physiol.*, 1937, **120**, 167.

[4] Pfeiffer, C. A., *Am. J. Anat.*, 1936, **58**, 195.

TABLE 1

Incidence of Maternal Behavior (Retrieving) in Normal and Experimental Mice.

Sex	Condition of the animal	Age in days	Sensitization with young*	No. of animals		No. of tests		% of positive tests
				Tested	Found maternal	Total	Positive	
F	Before opening of the vagina	23-35	++++	5	4	21	8	38
F	Virgins	36-180	++	9	8	39	22	56
F	First lactation	60-148	—†	15	15	132	114	86
F	2nd-3rd lactation	116-179	—†	5	5	35	30	86
F	4-24 weeks after last parturition		—	12	10	38	35	92
M	Before descent of testes	22-28	++++	8	7	40	19	47
M	Normal	36-120	++	56	47	162	103	63
M	Castrated at birth	35	+++	2	2	11	5	45
M	Castrated after puberty	70	++	3	2	12	5	42
M	Ovarian grafts	150	—	3	2	14	5	36
M	10,000-12,000 R.U. oestrin since birth	121-243	—	9	8	34	16	47

*Each + indicates presence of newborn young in the cage for one day.

†During lactation there is stimulation by the animal's own young.

Hormonal factors must also play a rôle because, as analysis of the data shows, there is an increase in maternal instinct at the end of the first pregnancy without contact with young other than that afforded by testing. However, during the lactation period no variations of maternal behavior, such as reported for the rat,[5] were observed in the mouse. Later on, either during subsequent lactations, or 6 to 24 weeks after the last parturition, maternal behavior is maintained at the same high level. In the latter case, neither the uninterrupted presence of young nor lactation hormones, but only a nervous mechanism could account for the maintenance of maternal behavior.

The presence of maternal behavior in males castrated at birth shows that this behavior does not require previous action of the sex (gonadal) hormones. Finally, the positive results with hypophysectomized animals[3] demonstrate that hypophyseal hormones are not necessary for maternal behavior. In rats, similar positive results have been obtained with virgin females, normal young males, and young castrated males.

Conclusion. The pattern of maternal behavior includes (a) retrieving, licking, and cuddling of the young; and (b) nest-building, prolonged station on the nest, and defense reactions. The initiation and maintenance of the latter group of activities are due to various physiological factors; for instance, temperature regulation[1] and thyroid function[2] account for nest-building, while decrease of activity[8] is at least in part responsible for prolonged station on the nest.

Retrieving, licking, and cuddling of the young, which may be considered as the main features of maternal behavior, may be initiated and maintained without hormonal intermediaries. The mere presence of young in the cage of the animals for some time is sufficient to bring about maternal behavior, even in hypophysectomized animals. Evidently nervous mechanisms play the determining rôle.

In addition to nervous factors, hormonal actions may be detected just before, and possibly during the first lactation period. However, the absence of measurable variations in maternal behavior during and after the first lactation period, as well as the presence of this behavior in males and females under various normal and experimental conditions, show that neither gonadal nor hypophyseal hormones play a primary rôle in the mouse.

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[5] Nissen, H. W., *J. Gen. Psych.*, 1930, **37**, 377; Wiesner, B. P., and Sheard, N. M., *Maternal Behaviour in the Rat*, London, 1933.

[6] Rogers, F. T., *J. Comp. Neurol.*, 1922, **35**, 21; Noble, G. K., *Anat. Rec.*, 1936, **64** (supp.), 34.

[7] Coghill, G. E., *Anatomy and the Problem of Behaviour*, Cambridge, 1929.

[8] Slonaker, J. R., *Am. J. Physiol.*, 1925, **71**, 36.

The author wishes to express appreciation to Dr. W. U. Gardner for some of the experimental animals (ovary-grafted males and oestrin-injected males) and to Dr. H. W. Nissen for suggestions and correction of the manuscript.

9743

Distribution of Chloride in the Gastric Mucous Membrane of the Dog.

I. GERSH. (Introduced by C. G. Hartman.)

From the Department of Anatomy, Johns Hopkins University.

An improved histochemical method for the demonstration of chloride was employed in a study of the dog's gastric mucous membrane under different physiological conditions. It was hoped that with this sensitive method it would be possible to throw some light on the site and mode of formation of hydrochloric acid in the gastric juice. A recent reconsideration of the problem by Hoerr[1] confirmed the theory developed by Harvey and Bensley[2] 25 years ago. These workers believe that hydrochloric is formed in the outer portion of the lumen or in the foveolus of the gastric glands by the hydrolysis of some organic chloride formed, stored and secreted by the parietal cells. In support of this theory chloride in large amounts has been demonstrated in these cells by methods which, however, have been considered by some investigators to be of questionable value.[3] Opposed to this theory are the findings of Lopez-Suarez[4] and Lison[5] who claim that the parietal cells contain less chloride than the other types of cells in the fundic glands. These opposing views emphasize the need for a reinvestigation, with more reliable methods, of the distribution of chloride in the gastric mucous membrane.

The method used in this investigation for the histochemical identification of chloride[6] is believed to visualize this ion accurately very nearly in the position which it occupies during life. Small pieces of gastric mucous membrane of dogs are carefully freed of the external coats of smooth muscle, and frozen in liquid nitrogen. They are dried in a vacuum chamber maintained at —60°C. or at —62°C. After warming to room temperature to avoid the condensation of water vapor, they are embedded in paraffin (m.p. 52°C.). Sections of this material are passed through petroleum ether, and are treated with a drop of acidified concentrated silver nitrate. The reagent is replaced with a drop of glycerine, and the section is reversed on a slide section-side down. The slide is exposed to carbon-arc radiation for a short time and observed directly thereafter. Depending on the amount present, chloride appears as a yellow to brown or black color which is resolved on greater magnifications into a lesser or greater number of particles of reduced silver. Properly carried out, this method is sensitive, specific and almost free from postmortem diffusion errors.

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[1] Hoerr, N. L., *Anat. Rec.*, 1936, **65**, 417.

[2] Harvey, B. C. H., and Bensley, R. R., *Biol. Bull.*, 1912, **23**, 225.

[3] Lison, L., *Histochemie Animale*, Paris, Ganthies-Villers, 1936.

[4] Lopez-Suarez, J., *Biochem. Z.*, 1912, **47**, 490.

[5] Lison, L., *Z. f. Zellforsch u. mikr. Anat.*, 1936, **25**, 143.

[6] Gersh, I., *Anat. Rec.*, 1938, in press.

Sections were prepared in this way of gastric mucous membrane at "rest" and during activity. The "resting" phase was studied in the mucosa of dogs deprived of food for one day. The active phase was analyzed in the glands of dogs 40 minutes after feeding a meat diet, after the subcutaneous injection of histamine, or after both feeding and the administration of the drug. The animals were killed by bleeding under ether anesthesia.

The distribution of chloride in the resting gland was found to be very characteristic. Chloride was absent from the cytoplasm of all gland cells. Traces of this ion were identified, however, in the secretion granules of the zymogenic cells. Chloride was present in the lumen of the gland along the free border of the cells, it was present in greater amounts in the foveolus and on the surface epithelium. Chloride was evenly distributed throughout the connective tissue spaces of the lamina propria mucosa and the tela submucosa.

The distribution of chloride in the actively secreting mucous membrane did not differ essentially from that in the resting tissue. In the active glands chloride was demonstrable in the same sites which it occupied in the resting glands, the only difference being that it was present in greater amounts. More chloride was visible in the cytoplasmic inclusions of the zymogenic cells, on the free surface of the epithelial cells, and in the tissue fluid spaces of the connective tissue layers. The cytoplasm of all the glandular cells remained free of chloride.

The failure to visualize chloride in the cytoplasm of the glandular cells leads one to assign these cells to the same category as skeletal muscle fibers which are also believed to be free of this ion. The presence of chloride in the secretion granules of the chief cells, if it is not caused by some unknown source of error, is unexplainable at the present time. The increase in the amount of chloride in the lumen and on the surface of the active mucous membranes may be attributed to increased amounts of hydrochloric acid secreted during digestion. In the same way, the prominent increase in the tissue fluid space may be correlated with greater amounts of chloride-containing tissue fluid and lymph that are formed during glandular activity.

It must be pointed out that there appears to be a very definite conflict betwen the conclusions derived from neutral red studies on the gastric mucosa and the more recent studies on the distribution of chloride in the epithelium. This conflict may be resolved, at least to some extent, by the possibility that the method employed cannot visualize a relatively non-reactive, not easily hydrolyzable protein chloride. Were this the case, it would be difficult to understand how the protein chloride could be hydrolyzed in the lumen or foveolus of the gastric glands by simple dilution in a manner advocated by Bensley, Harvey and Hoerr. A second possible way of removing this difficulty is that the protein chloride is extruded from the parietal cell almost as rapidly as it is formed, with no apparent storage of the compound in the cytoplasm. In this case, then, the resting and the active secretion phases differ only in their rate of the formation and extrusion of this protein chloride, all other processes remaining unaltered. However, it must be noted that if the distribution of chloride is really in fact that described in this paper, it does not implicate the parietal cell in the formation of hydrochloric acid any more than it involves any other cell type. By the same token, it does not exclude the parietal cell as the one whose specific activity results in the secretion of hydrochloric acid or its precursor.

9744

Changes in Blood Density in Adult Man in Acute Fever Induced by Typhoid Vaccine and Malaria.

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The experiments herein reported were designed to study the possible changes of blood anhydremia that might be associated with acute paroxysms of fever in adult man. That water plays an important rôle in the regulation of body temperature has long been recognized in both experimental and clinical work. The relationship is, however, a complicated one. Dehydration fevers have been produced experimentally and are recognized clinically. Excessive thirst is a frequent concomitant of fever and the administration of adequate amounts of water is a recognized factor in the treatment of varied types of fever. The details of the regulation mechanism of water distribution, water content and body temperature seem to vary widely in different species of homothermous animals, and in man it may vary with many factors, such as age, body weight, surface area, character of skin covering, etc. With these considerations in mind, it seems obvious that the analysis of the details of the mode of temperature regulation in a given animal species does not necessarily apply to that of a different species of homothermous animal, or even of the same animal at different stages of its life cycle.

Barbour and his associates have presented many cogent contributions suggesting that blood concentration is an essential factor in temperature regulation against cold and that blood dilution is a factor in regulating against heat. His method of measuring the density of the blood reveals these changes in the peripheral blood. From these changes in density he has calculated the volume changes of hydration or dehydration of the blood necessary to produce the density change. To a certain limited extent, therefore, changes in blood density may reflect changes in blood volume. It is obvious to all students of temperature regulation that there are many compensating factors that may mask the relation between blood solids and blood volume: *e. g.*, the addition or withdrawal of erythrocytes to or from the red cell reservoirs, alterations of chloride excretion to maintain chemical composition of hydremic blood, etc. But with all due allowance for these factors, it seems that acute changes of blood hydration can be detected by alterations of blood density.

With these considerations in mind we have studied the changes in blood density before and during the onset of acute fever in adult men induced by the intravenous injection of typhoid vaccine and during the acute chills of malaria. These tests have been made on 4 subjects, all males between the ages of 36 and 45 years. These men were in good physical condition and were being treated for central nervous system lues.

Ten determinations of blood density by Barbour's method[1] were made before and after the injection of typhoid vaccine. In each instance the following standard routine was followed: No breakfast was given, no fluids were taken by mouth after 10 A. M. at which time an adequate dose of typhoid vaccine was injected into the cubital vein. Blood was taken from either the finger tip or from the vein at intervals of 30 minutes to one hour, before the onset of fever, during, and after the fever reaction. All temperatures are oral.

Within 30 minutes after the injection of the vaccine there occurred uniformly an increase in blood density. Uniformly in all determinations the increased change of density precedes the rise of body temperature. Furthermore, the increased density of the blood persists for only a short time and may return to the initial level, even though the body temperature continues to rise. The temperature may remain elevated for several hours after the density has fallen to its original level or lower. (Chart I.)

CHART I.

Four Determinations of Finger Tip Blood Density During Onset of Chill and Fever Induced by Typhoid Vaccine, Given Intravenously. Adult Man.

Time	Body Temp.	Blood Density	Body Temp.	Blood Density	Body Temp.	Blood Density	Body Temp.	Blood Density
10	98.4	1.0586	98.2	1.0530	98.0	1.0530	96.5	1.0548
10:30	98.4	1.0602			98.2	1.0536		
11	98.4	1.0621	98.2	1.0548	98.6	1.0538	96.6	
11:30	101.5	1.0625			100.0	1.0542	96.8	1.0560
12	104.6		99.2	1.0556			97.0	1.0563
12:30	104.0	1.0612			102.2	1.0526		
1			99.8	1.0530			99.5	1.0566
1:30								
2			101.0	1.0532	102.0		99.8	1.0542
2:30	102.0	1.0590						
3					101.6		100.0	
3:30								
4					102.0	1.0528		

In Fig. 1 are shown simultaneous determinations of the density of finger blood and blood from the cubital vein. These determina-

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[1] Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, 1926, **69**, 625.

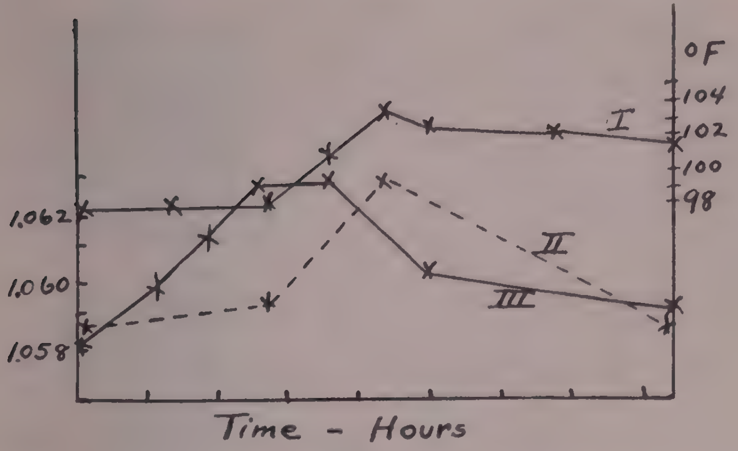

Fig. 1.

Body temperature in °F. shown by curve I. Density of finger tip blood shown by curve III. Density of venous blood by curve II.

tions were made to determine whether or not the density changes were confined to the capillary blood or were also exhibited by venous blood. It will be observed that the same increase in density occurs in the venous blood as in finger tip blood, but it is shortly delayed in time. Routine counts of red blood cells made during the onset of fever showed no significant change. There is a slight lowering of the carbon-dioxide combining power during the chill and onset of fever.

A similar study was made on 2 men preceding, during, and after the chill of malaria (Chart II). In this instance it will be noted that there is an increase in the density of the blood during the chill when the temperature is rising, but when the chill and muscular spasm is over, the blood density falls to a normal level or even below, although the fever is rising and remains elevated for several hours. With the onset of sweating and decline of the fever an increase in blood density may appear.

These findings indicate that in the onset of fever in the adult man, induced by typhoid vaccine or by malaria, there is increased density of the peripheral blood during the preliminary chill when the body temperature begins to rise. This, however, is a short reaction of less than 2 hours' duration. The blood density is then reduced to a normal or subnormal level, although the body temperature may remain elevated for several hours thereafter. It is, therefore, evident that the maintained state of fever induced by

CHART II.
The Changes of Blood Density Associated with the Chill and Fever of Malaria.

Time	Pulse	B.P.	Body Temp.	Blood Density	Comment
Mr. E. T. B., age 36. CNS Lues. Date 6-16-36					
10 A.M.	68	100/65	98.0	1.0505	Breakfast at 7 A.M. Resting in bed quietly
10:35			98.2		
11	88		98.4		
11:25	86	90/65	98.2	1.0511	1 glass tepid water
12:30	90		98.6	1.0508	
1:05					Chill begins
1:10	140			1.0515	Pallor—chill
1:30	112	115/70	102.0	1.0525	Chill is ending
2	116		104	1.0511	
2:30			104	1.0496	
3					Delirium
4			103.6	1.0499	
5:30			102.0		
Mr. H. A. M., age 42. CNS Lues. Date 6-25-37.					
9 A.M.	86	95/65	98.0	1.0552	Breakfast at 7 A.M.
11	92	100/70	100.0	1.0549	
3:45 P.M.	106	110/60	103.4	1.0565	Chill—blankets
4:15	120		103.6	1.0543	Water by mouth
5	118		104.0	1.0551	
5:40	120		104.6		Water by mouth
6:15	120		105.0	1.0551	Chill—blankets
8:15	116	105/55	103.8	1.0550	Fruit juice by mouth
10:15	104		100.6	1.0592	Patient is sweating freely
8 A.M.	92		99.0		

these means is not dependent on the concentration, dehydration or anhydremia of the blood itself. If the reduction in blood density is evidence of dilution of the blood, then during the fevered state there is really hydremia of the blood stream which, according to Barbour, is a normal method of reacting to heat.

The study of Soule, Buckman and Darrow[2] on blood volume changes during fever in children indicates that there usually occurs an increase in plasma volume. Our present study on the adult man is in harmony with this conclusion, with the additional finding that in the onset of acute fever with a chill, blood concentration precedes the rise of temperature but this is quickly followed by a lowering of blood density as the fever persists.

Since this work was done we have observed in work done by Black[3] that hay-fever paroxysms induced in a sensitive individual by intranasal injection of ragweed extract is also preceded by a sudden, sharp, but brief, increase in the density of blood taken from the finger. This is apparently due to the vasoconstricting effect of the allergic reaction.

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[2] Soule, H. C., Buckman, T. E., and Darrow, D. C., *J. Clin. Invest.*, 1928, **5**, 229.

[3] Black, J. H., *J. Allergy*, 1936, **8**, 39.

The above findings suggest that the increase in blood density which precedes the acute paroxysm of fever with chill, muscle spasm, pallor and peripheral constriction is probably to be considered as a part of the anaphylactoid reaction of the fever-producing agent rather than an essential factor in the mechanism of fever itself. After the first 2 hours of fever, there is a state of blood dilution. With the termination of the fevered state by sweating, blood concentration may again appear.

9745 P

Synthesis of Hippuric Acid in Man Following Intravenous Injection of Sodium Benzoate.

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With the increasing employment of the synthesis of hippuric acid as a means of determining liver function, several disadvantages of this test have been recognized. Vomiting occasionally occurs after the ingestion of sodium benzoate; the collection of urine for 4 hours is at times inconvenient; and in rare instances the condition of the patient may not permit the oral administration of the drug.

To meet these difficulties, the intravenous injection of sodium benzoate was investigated. 1.77 gm. of sodium benzoate (equivalent to 1.5 gm. benzoic acid) dissolved in 20 cc. of distilled water were given intravenously. The subject voided before the test began and a complete urine specimen was collected exactly one hour after the completion of the injection. Approximately 5 minutes were required for the injection. The hippuric acid was determined by the senior author's simple clinical method.[1] The results obtained on normal adult subjects are given in Table I.

From these results one can conclude that after the injection of 1.5 gm. of benzoic acid (as the sodium salt), 0.7 to 0.95 gm. are excreted as hippuric acid during the first hour by a healthy adult subject. If exogenous glycine is supplied, the synthesis of hippuric acid is greatly increased, which indicates that the amount of hippuric acid excreted is a measure of the body's capacity to synthesize glycine.

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[1] Quick, A. J., *Am. J. Med. Sci.*, 1933, **185**, 630.

TABLE I.

Subject	Hippuric acid (expressed as benzoic acid) excreted in hour	Remarks
1	.71	Test done a.m.
	.73	Test done p.m.
	1.01	15 gm. gelatin ingested 1 hr. before test
2	.95	
	.91	
3	.76	
	.88	
4	.84	
	.92	
5	.92	
6	.72	
7	.99	
8	.82	
9	.81	
10	.79	
11	.74	
12	.82	
13	.91	
14	.74	

The intravenous injection can therefore serve as an alternative method to the oral administration of sodium benzoate in the determination of liver function by means of the hippuric acid synthesis.

9746 P

Acetylation of Sulfanilamide by Liver Tissue *in vitro.*

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Marshall, Emerson and Cutting[1] and Fuller[2] have demonstrated that sulfanilamide is partially converted by man and the rabbit into a conjugated form. The latter has been isolated from the urine of these species after oral ingestion and has been shown to be acetyl sulfanilamide. Ellinger and Hensel[3] have demonstrated the acetylation of para-amino-benzoic acid by the rabbit, and Muenzen, Cerecedo, and Sherwin[4] have concluded that acetylations are very likely confined to the liver.

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[1] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C., *J. Am. Med. Assn.*, 1937, **108**, 953.

[2] Fuller, A. T., *Lancet*, 1937, **1**, 194.

[3] Ellinger, A., and Hensel, M., *Z. physiol Chem.*, 1914, **91**, 21.

[4] Muenzen, J. B., Cerecedo, L. R., and Sherwin, C. P., *J. Biol. Chem.*, 1924, **60**, XVI.

In an attempt to elucidate the course and site of the acetylation of sulfanilamide, tissue slices of rabbits and rats were suspended in Ringer bicarbonate solution (pH 7.4) containing 0.2% glucose and concentrations of sulfanilamide varying from 10 to 200 mg. %. The mixture was then shaken in a water bath at 37°C. for 4 to 5 hours in an atmosphere of 5% CO_2 and 95% O_2. Samples of the fluid were withdrawn and analyzed for free and conjugated sulfanilamide by the method of Marshall.[5] Of the tissues used (liver, muscle, spleen, kidney, and blood) only the liver could convert sulfanilamide into the conjugated form, and of the preparations used (slices and broken cell suspensions) only the slices were effective. The conjugated form was isolated from the mixture in a crystalline state and was shown to be acetyl sulfanilamide by mixed melting point determinations and by the recovery of the theoretical amounts of acetic acid and sulfanilamide after acid hydrolysis. Conjugation was observed with each of 14 rabbit livers, but with only 2 of 4 rat livers.

The addition of sodium lactate, pyruvate, acetate, and aceto-acetate increased the aerobic formation of the conjugated compound. Under anaerobic conditions, little or no acetylation took place except in the presence of added acetate or pyruvate.

Although the acetylation of sulfanilamide is thus dependent upon aerobic respiration, it was found, by the Warburg technique[6] that the presence of sulfanilamide in concentrations up to 200 mg. % had no effect upon either aerobic or anaerobic tissue respiration.

The extent of the conversion under varying conditions is indicated in Table I.

TABLE I.
Mg. Sulfanilamide Converted into Conjugated Form per Mg. of Dry Tissue

Original conc. of sulfanilamide in mg.%	Control	Acetate	Pyruvate	Lactate	Acetoacetate
		Aerobically.			
20	.0059	.0068	.0081	.0082	.0068
50	.0063	.0148	.0113	.0133	.0112
200	.0060	.0097	.0193	.0082	.0133
50	.0075	.0146	.0139		
		Anaerobically.			
20	.0022	.0040	.0084		
50	None	.0048	.0043		
20	”				
20	”				

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[5] Marshall, E. K., Jr., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 422.

[6] Warburg, O., *The Metabolism of Tumors*, Chapter 5, page 75, Constable and Co., Ltd., London, 1930.

The final concentration of added substances ranged from 0.02-0.04 M. 50 to 150 mg. wet rabbit liver slices were used per 3 cc. medium.

9747 P

Observations on Histologic Structure of Anterior Pituitaries of Old Female Rats.*

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Considerable information is now available concerning the histologic structure of the anterior pituitary of the female rat during the normal estrous cycle,[1] and after various experimental procedures.[2, 3] However, as far as the writers are aware, there are available in the literature no reports of definite histologic studies on the anterior pituitaries of old female rats. Below are presented certain observations on a histologic condition found in the anterior pituitaries of old female rats which we have not observed in the anterior lobes of younger animals.

We have examined the pituitaries of 26 old breeding female rats of the experimental colony strain of the Wistar Institute. A majority had given birth to from 4 to 6 litters; all had delivered at least one litter. At autopsy the animals ranged in age from 543 to 848 days, the average age being 632 days. Three animals were found to have small subcutaneous tumors in the mammary region; subsequent histologic examination revealed that these were fibroadenomata of the mammary gland. The pituitaries were variable in weight, ranging from 10 to 45 mg., the majority weighing less than 17 mg. In 2 animals the right lateral halves of the anterior lobes were markedly and irregularly enlarged, causing a definite anterior and dorsal bulging of the anterior lobe tissue. The enlarged areas were extremely congested and very soft. In both, the posterior lobe appeared to be compressed and reduced in size. These enlarged

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* These studies have been aided by a grant from the International Cancer Research Foundation.

[1] Wolfe, J. M., and Cleveland, R., *Anat. Rec.*, 1933, **55**, 233.

[2] Severinghaus, A. E., *Physiol. Rev.*, 1937, **17**, 556.

[3] Wolfe, J. M., *Albany Medical Annals*, 1937, **56**, 102.

areas were thought to be adenomata of the pituitary. In 2 other animals small, markedly congested, papilla-like enlargements of anterior lobe tissue projected up from the dorsal surface of the gland.

Serial sections of all pituitaries were made and studied by methods previously described.[1] In 15 rats, 58% of those studied, were found localized areas of cells which were histologically different from those making up the normal portions of the gland. In some glands these areas were small, containing only a few cells. In most cases, however, they were larger, measuring from 0.5 to 1.0 mm. in their greatest dimension. In 2 animals they constituted practically all of the lateral half of the gland.

The cells in these areas differed from the normal cells in that they were generally markedly hypertrophied. Cells as large as 40 x 50 micra in size were sometimes observed. A majority of these enlarged cells were considered to be chromophobes but in some glands enlarged eosinophiles were also found. In the hypertrophied chromophobes, the nuclei were large and as a rule appeared to contain little chromatin. The nucleoli were very large and prominent. The negative image of the Golgi apparatus was greatly increased in size. The cytoplasm varied in color from a dense blue to a light blue; that of the latter cells was often fragmentary. Many cells contained varying numbers of small clear vacuoles in the cytoplasm. In the enlarged eosinophiles the negative image of the Golgi apparatus was greatly increased in size; most cells exhibited a reduced granular content.

In the smaller areas of hypertrophied cells, the cells were usually closely packed together and there was little dilatation of the capillaries. However, in the large areas the capillaries were extremely dilated in some instances, throwing the anterior lobe cells into narrow cords separated by wide sinusoidal-like blood spaces. In the regions of marked capillary dilatation the degree of hypertrophy of the cells was seldom as great as in those regions where the blood supply was less abundant. In the 2 animals in which adenomata were diagnosed grossly, the whole lateral halves of the glands were made up of chromophobes showing varying degrees of hypertrophy. The capillaries were markedly dilated. Large masses of intercellular colloid were found throughout the anterior lobe tissue. There was a compression of the normal anterior lobe tissue which surrounded the area of the hypertrophied cells. We have tentatively regarded these areas as adenomata of the pituitary, although it has not been definitely established that they possess all the characteristics of true tumors.

These studies indicate that in old rats of this strain there is a tendency for atypical areas of hypertrophied cells to appear. The cells in these areas are histologically similar to those found in the adenomata of the pituitary described above.

9748

Observations on Dolman's Test for Determining the Presence of Staphylococcal Enterotoxin.

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Numerous observations indicate that staphylococci are frequently present in cases of food poisoning. It is often difficult, however, to determine whether or not this organism is the etiological agent. The studies of Jordan and McBroom,[1] Woolpert and Dack,[2] and Dolman, Wilson and Cockcroft,[3] suggest that toxin produced by staphylococci is responsible for the clinical symptoms. Little is known about the pathological lesions in cases of food poisoning resulting from staphylococci because only one death has been reported.[4]

Dolman and his associates[3] published a method by which they could determine whether or not the staphylococcus found in the food in cases of poisoning is the etiological agent. Furthermore, with this test they showed that the gastro-intestinal fraction in staphylococcal toxin was not destroyed by heat for 20 minutes at 100°C. The thermolability of this fraction is very different from that of the hemolysin, leucocidin and skin-necrotizing fractions. The technic of this test consists in injecting intraäbdominally into kittens toxin prepared from the cultures of staphylococci. If the animal shows marked lassitude and weakness, with unsteadines which appears shortly after the injection and if these symptoms culminate in the first of a series of intermittent paroxysms of vomiting, associated with diarrhea, then the toxin contains a gastro-intestinal poison.

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[1] Jordan, E. O., and McBroom, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 161.

[2] Woolpert, O. C., and Dack, G. M., *J. Inf. Dis.*, 1933, **52**, 6.

[3] Dolman, C. E., Wilson, R. J., and Cockcroft, W. H., *Canad. Public Health J.*, 1936, October, 489.

[4] Blackman, S. S., *Bull. Johns Hopkins Hosp.*, 1935, **57**, 289.

Stone[5] has recently suggested a bacteriological method for distinguishing staphylococci that produce a gastro-intestinal toxin. The results of Dolman and his associates differ from those of Stone.

I have been unable so far to separate satisfactorily the different components which have been described as occurring in staphylococcal toxin. Dolman's test seemed to offer a satisfactory method to differentiate the gastro-intestinal from the other fractions in one of the toxin-preparations I have been studying.

The toxin* was prepared by a method very similar to that used by Dolman. The control was the uninoculated medium.* This medium was centrifuged and the supernatant fluid was used for the inoculations. Puppies weighing from 1500 to 2500 gm. and kittens from 750 to 1150 gm. were injected intraäbdominally. The toxin was heated at 100°C. for 2 hours.

Two puppies were given 5 cc. of the heated toxin and 2 were given 10 cc. Three puppies were given 10 cc. of the control medium. The majority of these animals were drowsy for approximately 4 hours thereafter. None inoculated with heated toxin vomited. Each of the 3 given the control medium vomited one or more times during the first 30 minutes of the experiment. All the 7 puppies appeared normal after 18 to 24 hours except one which had a diarrhea.

These puppies were again injected after 24 hours with the following preparations: Five were given 5 cc. of the control medium and 2 were given an equal quantity of staphylococcal toxin. All were sick immediately after the inoculation. Four of the 5 receiving the control medium vomited several times during the first 30 minutes. The fifth puppy never vomited. Both dogs given the toxin vomited within a period of one hour and one developed severe abdominal cramps and a diarrhea. After 4 hours the group given the control medium appeared normal except for one which had loose stools. Both of the puppies inoculated with the toxin were dead after 4 hours. The pathological changes in these were similar to those previously described following the intravenous injection of staphylococcal toxin.[6]

Four kittens were given intraäbdominally 5 cc. of the control medium and 2 vomited within a period of 30 minutes. Three were inoculated with 5 cc. of staphylococcal toxin and each vomited within 5 minutes. Three kittens were given a similar quantity of the toxin after it had been heated for 2 hours at 100°C. and none vomited. A majority of the kittens were drowsy and weak after each of the inoculations. One that received the control medium had a diarrhea after 24 hours. The 3 kittens given the staphylococcal toxin grew progressively weaker and all were dead after 4 hours. The pathological changes were similar to those in the puppies.

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[5] Stone, R. V., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 185.

* Supplied by Lederle Laboratories.

[6] Rigdon, R. H., *Arch. Path.*, 1935, **20**, 201.

The fact that the kittens and puppies were sick, vomited, and had a diarrhea following the intraäbdominal injection of 5 cc. of staphylococcal toxin indicates, according to Dolman's test, the presence of an enterotoxin. Three kittens given 5 cc. of heated toxin and 2 dogs given an equal volume of the same toxin and 2 other dogs given twice this amount did not vomit. Four out of a group of 5 puppies given 5 cc. of the control medium vomited within a period of 30 minutes. Of a group of 7 kittens given the control medium, 2 vomited.

Since some of the kittens and dogs in this study were sick and vomited following injection of the control medium it would appear impossible to determine whether or not they vomited from the effect of the toxin or from the effect of the medium. Furthermore it does not seem correct to conclude that staphylococcal enterotoxin is thermostable. In this study none of 4 dogs or 3 kittens vomited after receiving the heated toxin.

The volume of the material injected and the individual variation in animals of the same species may account for the observations that have been made in an attempt to separate the staphylococci into 2 groups: those which do and those which do not produce an enterotoxin.

9749 P

Cataphoretic Separation of Toxic Components of Moccasin Venom.

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Attempts at separation of the various active components of snake venoms have not been very successful. These principles are probably proteins or substances closely related to proteins.[1] They are quite unstable.[1, 2] For this reason, chemical procedures used in their separation, such as precipitation, extraction or absorption often resulted in the destruction of the less stable elements.

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[1] Peck, S. M., and Marx, W., publication in preparation.

Separation of the hemorrhagic and hemolytic components of moccasin venom by cataphoresis was attempted because it was thought that this procedure might be less destructive. This method had been tried by others without success in an attempt to separate the neurotoxic and coagulant principles of Bothrops venom[3] and the neurotoxin and hemolysin of cobra venom.[4]

In the present studies, a cataphoresis chamber was used as described by Todd.[5] An electrical potential of 120 volts D.C. was applied to solutions of moccasin venom for 3 to 5 hours at different hydrogen ion concentrations. The liquids in the anode and cathode chambers were then tested for hemorrhagin and hemolysin.

The hemorrhagin was assayed by using the intradermal venom test in rabbits.[2] It was found that at pH values of 6 and above, the hemorrhagin migrated only to the anode, below pH 4 only to the cathode. Very little migration occurred in the neighborhood of pH 4-5. This indicated that the isoelectric point of the hemorrhagic component of moccasin venom was between pH 4 and 5.

The assay for hemolysin was carried out on agar blood plates.[2] This test was recently improved by using plain agar instead of agar plus beef broth as medium.[1] With this improved blood plate method it was possible to show that moccasin venom contained 2 different hemolytic components, and to demonstrate their separation. They were designated as hemolysin A and B.

Hemolysin A was identical with the hemolytic component which had been discussed in our first publication.[2] This caused the appearance of dark olive to black areas of lysis on blood plates. It was quite unstable and was rapidly destroyed outside of the pH range of 5-7. Hemolysin A was found to have an isoelectric point of approximately pH 5. At pH 6 and 7 it migrated only to the anode during cataphoresis.

Hemolysin B, the other hemolytic component, differed markedly in its lytic properties from hemolysin A. When the original blood plate method for demonstrating hemolysin was used, its hemolytic action was hardly visible. For this reason it was not discussed in our first report.[2] By the use of the improved blood plate method, however, its action could readily be demonstrated, but only in the absence of hemolysin A. The action of hemolysin B became manifest as a colorless transparent spot of lysis on the blood plate. During cataphoresis, hemolysin B always migrated to the cathode, independently of the pH, in the studied range from 3 to 8.

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[2] Peck, S. M., and Marx, W., *J. Pharm. Exp. Therap.*, 1937, **60**, 358.

[3] v. Klobusitzky, D., *Arch. Exp. Path.*, 1935, **179**, 204.

[4] Ghosh, B. N., and De, S. S., *Ind. J. Med. Res.*, 1937, **24**, 1175.

[5] Todd, C., *Brit. J. Exp. Path.*, 1927, **8**, 369.

Since hemolysin B could not be demonstrated by the blood plate method in the presence of hemolysin A, a different technique had to be employed for its determination. This consisted in the titration of the minimal hemolysin concentration which produced complete hemolysis of a suspension of rabbit blood cells under defined conditions, lecithin being used as activator. Lecithin only activated hemolysin B. The method will be described later.[1] By this means it could be confirmed that, during cataphoresis, hemolysin B migrated only to the cathode in the studied pH range. The anode liquid was practically always negative for hemolysin B when tested in this way.

It was thus possible to demonstrate that moccasin venom contained 2 different hemolytic components which could be separated by cataphoresis: At pH 6 and 7, hemolysin A migrated only to the anode, hemolysin B only to the cathode. It could also be shown that in the same pH range, the hemorrhagin and hemolysin B could be separated by cataphoresis. The hemorrhagin migrated only to the anode, the hemolysin B only to the cathode.

Our findings seem to indicate that the molecules responsible for the action of hemolysin B are different from those which give rise to the other toxic effects of moccasin venom so far studied. Hemolysin B has a much more basic character.

9750

Protection of Rabbits Against Naturally Acquired Infectious Myxomatosis by Previous Infection with Fibroma Virus.

Richard E. Shope.

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It is generally agreed that the viruses of rabbit fibroma and rabbit myxoma are closely related immunologically and that infection with the benign fibroma virus usually protects rabbits against fatal infection with the myxoma virus.[1–8] This suggested that the fibroma virus might make a satisfactory immunizing agent for practical use in the control of infectious myxomatosis. The possibility was tested by McKenney and Shillinger[9] with discouraging results, and they concluded that the infection of rabbits with fibroma virus did not constitute a satisfactory or practical means of immunizing against infectious myxomatosis. However, careful consideration of the experiments of McKenney and Shillinger suggests that their difficulties lay more in the maintenance of a potent infective fibroma virus for use in their experiments than in an actual failure of fibroma virus to enhance the resistance of rabbits to myxomatosis.

Partly because of McKenney and Shillinger's adverse report on the practicability of using fibroma virus prophylactically and partly to determine whether fibroma-recovered rabbits would prove as resistant when exposed to cases of infectious myxomatosis as they had when inoculated with myxoma virus, the experiment to be reported in this paper was conducted.

The testicles from a rabbit inoculated intratesticularly 7 days earlier with strain A fibroma virus were removed aseptically and stored for one month in 50% glycerol in the refrigerator. (The period of storage in glycerol is not important providing it does not exceed 2 months.) At the end of this time they were washed in 3 changes of physiological saline, ground with sterile sand and suspended in physiological saline to make a 5% suspension. After the suspension had stood for 10 minutes in a tall glass graduate, the supernatant fluid was decanted for use as fibroma virus.

Each of 7 rabbits received 0.5 cc. of the fibroma virus intradermally. Another 7 rabbits were inoculated by applying 0.5 cc. of the virus to a small area of shaven skin that had been freshly scarified by means of sandpaper. The virus was rubbed into the scarified area with the rounded closed end of a small sterile tube. Fibromas appeared at the sites of inoculation in all 14 rabbits, reached their maximal size in from 12 to 14 days and then regressed within a month.

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[1] Shope, R. E., *J. Exp. Med.*, 1932, **56**, 803.

[2] Hyde, K. E., *Am. J. Hyg.*, 1936, **23**, 278.

[3] Hyde, R. R., *Am. J. Hyg.*, 1936, **23**, 425.

[4] Berry, G. P., and Lichty, J. A., Jr., *J. Bact.*, 1936, **31**, 49.

[5] Hyde, R. R., *Am. J. Hyg.*, 1936, **24**, 217.

[6] Martin, C. J., Commonwealth of Australia, Council for Scientific and Industrial Research, Bull. 96, 1936.

[7] Shope, R. E., *J. Exp. Med.*, 1936, **63**, 43.

[8] Hurst, E. W., *Brit. J. Exp. Path.*, 1937, **18**, 23.

[9] McKenney, F. D., and Shillinger, J. E., *J. Am. Vet. Med. Assn.*, 1935, **87**, 621.

TABLE I.

Effect of Previous Infection with Fibroma Virus on Resistance to Infectious Myxomatosis.

Rabbit No.	Previous treatment	Result of pen exposure to infectious myxoma
1599	None—control	Died of myxomatosis—14th day after initial exposure
1601	” ”	” ” ” 18th ” ” ” ”
1602	” ”	” ” ” 19th ” ” ” ”
1603	” ”	” ” ” 16th ” ” ” ”
1606	” ”	” ” ” 17th ” ” ” ”
1607	” ”	” ” ” 13th ” ” ” ”
1608	” ”	” ” ” 15th ” ” ” ”
1612	” ”	” ” ” 17th ” ” ” ”
1613	” ”	” ” ” 15th ” ” ” ”
1629	Fibroma virus intradermally	No illness
1630	” ” ”	” ”
1631	” ” ”	” ”
1632	” ” ”	” ”
1633	” ” ”	” ”
1636	” ” ”	” ”
1637	” ” ”	” ”
1620	” ” on scarified skin	Left-sided blepharitis—survived
1622	” ” ” ” ”	No illness
1624	” ” ” ” ”	” ”
1625	” ” ” ” ”	” ”
1626	” ” ” ” ”	” ”
1627	” ” ” ” ”	” ”
1628	” ” ” ” ”	” ”

Thirty-three days after infection with fibroma virus the 14 rabbits, together with 9 normal rabbits, were placed in a large pen with a rabbit that had received myxoma virus subcutaneously 3 days previously. The inoculated rabbit subsequently developed clinically typical infectious myxomatosis and died on the 9th day following inoculation. It served as the initial source of myxoma virus in the pen. The later course of the disease among the exposed animals in the pen is depicted in Table I.

As shown in the table, all 9 of the control rabbits died of typical infectious myxomatosis between the 13th and 19th days following their initial exposure to a myxoma-infected rabbit. None of the 14 rabbits recovered from fibroma, on the other hand, died, and only one showed slight symptoms of infectious myxomatosis (left-sided blepharitis) in spite of prolonged exposure to the virus not only in the inoculated rabbit but in the 9 control animals as well. The experiment was terminated on the 33rd day, 2 weeks after the death of the last control rabbit.

It would appear from this experiment that rabbits recovered from infection with fibroma virus possess a high degree of resistance to infectious myxomatosis transmitted by contact.

9751 P

Quantitative Studies on the Replacement of Body Chlorides.*

Robert M. Bartlett, Dermid L. C. Bingham,† Svend Pedersen, Walter G. Maddock and Frederick A. Coller. (Introduced by Howard B. Lewis.)

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The dangers of excessive salt administration have been pointed out by several observers,[1-4] while others have called attention to

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* This study was aided by the Horace H. Rackham Fund for Graduate Studies, and the James and Elizabeth Inglis Fund for Surgical Research.

† Fellow of the Medical Research Council of Great Britain, Clinical Tutor in Surgery, Royal Infirmary, Edinburgh.

[1] Matas, R., *Ann. Surg.*, 1924, **79**, 643.

[2] Jones, C. M., and Eaton, F. B., *Arch. Surg.*, 1933, **27**, 159.

[3] Jones, C. M., Eaton, F. B., and White, J. C., *Arch. Int. Med.*, 1934, **53**, 649.

[4] Coller, F. A., Dick, V. S., and Maddock, W. G., *J. A. M. A.*, 1936, **107**, 1522.

the equally serious effects of depleted body chlorides.[5-8] In an effort to place saline administration on a quantitative basis a study was made of chloride replacement in surgical patients with hypochloremia.

The plasma chlorides were determined by the Wilson and Ball modification of the Van Slyke method, and each day the chlorine content of the urine and of any gastrointestinal tract loss other than in the stool was determined by the Volhard-Arnold method. All results were calculated in terms of sodium chloride. During the period of study the patients received nothing by mouth, chlorides and glucose being given intravenously in the form of isotonic solutions.

The theoretical amount of sodium chloride needed by these patients was based upon the following considerations. The average chlorine content of the human body is estimated to be 0.15% of the total body weight.[9] In terms of sodium chloride this is 0.248% of the total body weight. Thus in a 70 kg. man there are 173.6 gm. of sodium chloride. White and Bridge[10] showed in dogs that depletion of plasma chlorides by vomiting was accompanied by a fall in the chlorine content of the tissues. From this it is apparent that the chloride ion passes with comparative ease from one body fluid to another, and that the plasma chloride level is an index of the level of the tissue chlorides.

With these facts in mind, knowing the weight of the patient and the level of plasma chlorides after depletion, one should be able to calculate the number of grams of sodium chloride needed to restore the chlorine content of the body to normal (using 560 mg. % as normal plasma chloride level), as follows.

$$\text{Gm. NaCl needed} = \text{normal salt content of body} \times \text{\% depletion of plasma chlorides}$$

$$= 0.248\% \text{ of Wt. (gm.)} \times \frac{560 - \text{depleted plasma chloride level}}{560}$$

Table 1 shows the degree of accuracy of the above formula for calculating the grams of sodium chloride needed in patients with hypochloremia. In all cases except 2 (B.S. and L.D.) the difference

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[5] Walters, W., Kilgore, A. M., and Bollman, J. L., *J. A. M. A.*, 1926, **86**, 186.

[6] Elman, R., and Hartmann, A. F., *Arch. Surg.*, 1930, **20**, 333.

[7] Dragstedt, L. R., and Ellis, J. C., *Am. J. Physiol.*, 1930, **93**, 407.

[8] Gatch, W. D., Trusler, H. M., and Ayers, K. D., *Am. J. Med. Sci.*, 1927, **173**, 649.

[9] Sherman, H. C., *Chemistry of Food and Nutrition*, Fifth Edition, 1937, p. 242, Macmillan Co.

[10] White, J. C., and Bridge, E. M., *Boston Med. and Surg. J.*, 1927, **196**, 893.

between the sodium chloride given and the sodium chloride retained could be accounted for by the additional salt necessary to replace that lost from the gastrointestinal tract during the time of the study. In the case of B.S. 9.2 gm. of sodium chloride were lost in the urine, and further administration of salt failed to raise the plasma chloride level significantly. Thus it appears that this individual was unable to maintain a higher plasma chloride level than that first attained. In L.D., besides a 5.8 gm. loss through the gastrointestinal tract, 14.9 gm. of sodium chloride were excreted in the urine. By accident an excess of sodium chloride approximating the urine loss had been given.

TABLE I.
Replacement of Body Chlorides After Depletion.
Calculated optimal retention is computed on the basis of a normal plasma chloride level of 560 mg./100 cc.

Patient	Body Wt., kg.	Initial Plasma Cl, mg. NaCl/100 cc.	NaCl Given, gm.	NaCl Retained, gm.	Calc. Optim. Retention, gm.	Plasma Cl after replacement, mg. NaCl/100 cc.
J.W.	59.8	447	26.2	25.1	29.7	546
T.J.	72.7	427	40.6	40.6	42.7	564
W.P.	67.7	436	33.7	32.2	37.1	543
O.M.	63.6	404	55.8	40.9	43.8	559
B.S.	39.0	449	28.4	18.0	19.2	493
J.C.	65.5	479	27.5	23.5	23.4	586
D.C.	60.4	513	15.9	14.6	12.6	564
D.E.	58.1	345	101.1	56.9	55.2	606
L.D.	60.2	372	70.1	49.4	50.1	566
S.T.	72.6	436	42.3	40.9	39.8	554

Falconer and Lyall[11] stated that the total sodium chloride content of the body is probably about 90 gm. Our results indicate that the statement that 0.248% of the body weight is sodium chloride (173 gm. in a 70 kg. individual) is more nearly correct. Falconer and Lyall concluded that about 20 gm. (15-30 gm.) of salt are required on the average to produce an increase in the plasma chloride level of 100 mg. per 100 cc. They noted that marked variations occurred. These variations, we feel, are undoubtedly due to differences in body weight. We have found that for clinical purposes the administration of 0.5 gm. of sodium chloride per kilo of body weight for each 100 mg. % that the plasma chlorides need to be raised gives satisfactory results. The amount provided by this calculation is slightly in excess of that calculated by the above formula, and this is an error in the right direction.

Summary. A simple formula is presented for determining the optimal sodium chloride retention to restore body chlorides to normal in patients with hypochloremia. On the basis of this formula it has been found that for clinical purposes the administration of 0.5 gm. of sodium chloride per kilo of body weight for each 100 mg. % that the plasma chlorides need to be raised may be expected to restore the body chlorides to normal.

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[11] Falconer, M. A., and Lyall, A., *Brit. Med. J.*, Dec. 4, 1937, p. 1116.

9752

Further Observations on Intranuclear Inclusions Produced by Non-Virus Materials.

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The purpose of this report is to record certain additional findings obtained in the application of the technic previously reported[1] for producing intranuclear inclusion-bodies with substances unassociated with a virus. This procedure consisted essentially of the injection of autoclaved materials subcutaneously into guinea pigs and the subsequent removal of nodules so produced for histological examination. When aluminum hydroxide or alundum was used, intranuclear inclusion-bodies were always found in the mononuclear and giant phagocytic cells of the tissue-reaction about one week after injection and usually as long as the nodules persisted. With ferric hydroxide and carbon they were found but irregularly or in small numbers. Histochemical reactions and morphological characteristics of such inclusions resembled very closely those of viral diseases. However, transmission and other experiments disclosed no virus in association with these inclusions.

Following is a list of additional materials which have been similarly injected subcutaneously into guinea pigs in order to determine whether the resulting tissue-reactions would also contain inclusions: suspensions of normal brain tissue of mice, of guinea pigs, and of rabbits (fresh or autoclaved); normal rabbit tissues (liver, spleen, kidney, testicle); commercial lecithin; alcoholic extract (lipins) of monkey brain; phosphatide of tubercle bacilli;* and 1:2:5:6 dibenzanthracene.† Although the reactions to these substances differed somewhat from one another and from that of aluminum hydroxide, they all contained mononuclear phagocytic cells and in certain instances also giant phagocytic cells. Of all the materials, only brain tissue gave reactions containing the characteristic intranuclear inclusions. These bodies were usually few in number and difficult to find, although some sections of the induced nodules contained moderate numbers while in others none were found. Inclusions occurred with rabbit and guinea pig brain, fresh or autoclaved, injected into guinea pigs but not with mouse brain. It was thought that the lipins of cerebral tissue might have caused the inclusions but the results do not show this; the constituent of this material which induces the bodies is still unknown.

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[1] Olitsky, P. K., and Harford, C. G., *Am. J. Path.*, 1937, **13**, 729.

* Kindly supplied by Dr. A. L. Joyner.

† Kindly supplied by Dr. A. Claude.

It has been shown by Smithburn and Sabin[2] that the phosphatide of tubercle bacilli, when injected into the connective tissues of guinea pigs, is ingested by phagocytic cells which can be identified by the supravital staining technic as epithelioid cells. As previously noted,[1] the cells that ingest aluminum hydroxide in the subcutaneous tissues of guinea pigs are also of the epithelioid variety, as determined by the supravital technic. The fact that the epithelioid cells in the phosphatide lesion do not show intranuclear inclusions, while those in the lesion of aluminum hydroxide do show them, indicates that these bodies in the nucleus are not merely characteristic of epithelioid cells in the subcutaneous tissue of the guinea pig but are associated with the presence of certain foreign substances.

That phagocytic cells in sites other than subcutaneous tissue may contain intranuclear inclusions after engulfing aluminum hydroxide is indicated by the following: Aluminum hydroxide has been injected intratesticularly into the peritesticular tissues, and into the pericardium of guinea pigs. Localized nodular masses were formed consisting of mononuclear and multinuclear phagocytic cells actively engulfing the foreign substance. The reactions resembled those in subcutaneous nodules, and the nuclei of the phagocytic cells contained similar inclusions. Although rabbits responded with the same type of tissue-reaction in the testicles as guinea pigs did, inclusions could not be found. Mice receiving aluminum hydroxide subcutaneously showed tissue-reactions similar to those of the guinea pig and the rabbit but in this animal intranuclear inclusion-bodies were seen only occasionally.

Dilutions of suspensions of aluminum hydroxide were injected subcutaneously into guinea pigs in order to make a rough determination of how small an amount would produce inclusions. The least amount to give a local reaction with inclusions was that equivalent to 0.01 mg. of Al_2O_3. Alundum is also a form of Al_2O_3 and is commonly used as an abrasive in the preparation of tissues for animal inoculation. In the usual procedure, however, it is completely removed from the tissue-suspensions by centrifugation.

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[2] Smithburn, K. C., and Sabin, F. R., *J. Exp. Med.*, 1932, **56**, 867.

Summary. The following non-virus materials were used in guinea pigs in addition to those already reported: suspensions of normal brain tissue; normal rabbit tissues (liver, spleen, kidney, testicle); commercial lecithin; alcoholic extract (lipins) of monkey brain; phosphatide of tubercle bacilli; and 1:2:5:6 dibenzanthracene. Of these only brain induced characteristic intranuclear inclusions. Aluminum hydroxide injected into other sites than the subcutaneous tissues of guinea pigs produced similar reactions with inclusions. The mouse responded only feebly with inclusions on injection of aluminum hydroxide while the rabbit again failed.[1] The approximate minimal effective dose of Al_2O_3 needed for production of the intranuclear bodies was found to be 0.01 mg. per cc. The various aspects of these findings are discussed.

9753 P

Relief of Myxedema and Cretinism by Iodinated Blood Serum.*

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Since Baumann's finding that thyroglobulin contains iodine, many investigators[1] have sought for the chemical mechanism by which hormonal activity is produced when the native protein is iodinated in the body. The isolations of (inert) diiodotyrosine and (active) thyroxine by hydrolysis of thyroglobulin, together with the formation of artificial thyroid protein from diiodotyrosine peptone[2] have suggested that under certain conditions the phenolic groups of diiodotyrosine may undergo conjugation to form an iodized thyronine nucleus. The rapid formation of active thyroid protein which occurs in glands of patients with exophthalmic goiter when iodine is

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* Aided by grants from the Ella Sachs Plotz Foundation and the International Cancer Research Foundation.

[1] Hutchison, R., *J. Physiol.*, 1898-99, **23**, 178.

[2] Salter, W. T., and Pearson, O. H., *J. Biol. Chem.*, 1936, **112**, 579.

administered suggests that its basis is a simple chemical mechanism which can be reproduced directly *in vitro.*

To this end we investigated the outcome of the hydrolysis of a nondescript iodoprotein so conducted as to favor condensation or synthesis. The procedure of Abelin[3] was modified to favor greater condensation (by mass-action) of split-products of hydrolyzed iodoprotein. The starting material was mammalian blood serum iodinated by the procedure of Wormall.[4] For each liter of blood serum there resulted about 1.2 gm. of material containing 24% iodine. From this a fraction containing 47% iodine was readily obtained, which approximates the composition of diiodothyronine (48%).

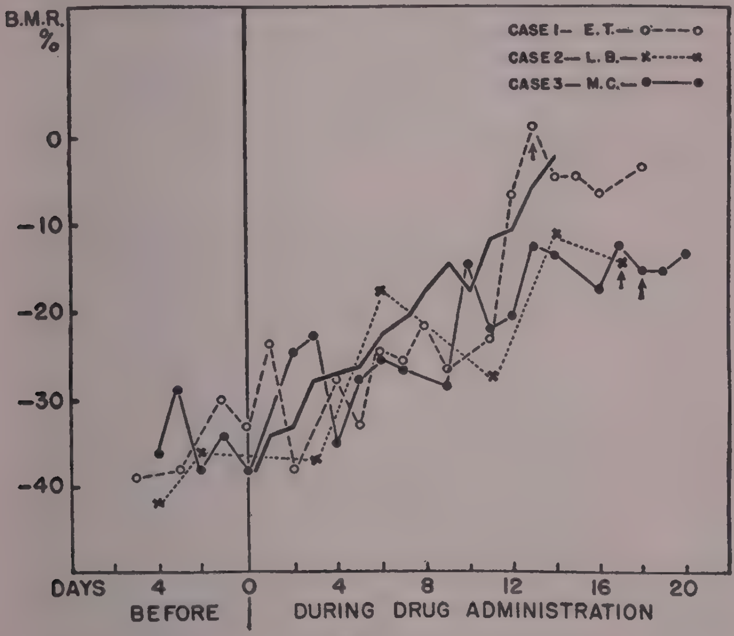

FIG. 1.

The metabolic response of 2 patients with myxedema and one with cretinism receiving the material described in the text. The arrows indicate omission of medication. The heavy solid line is our curve of reference and represents the metabolic response to the daily administration of thyroxin polypeptide containing 0.5 mg. of iodine.

Since human myxedema and cretinism offer the best test objects available for identifying thyroid activity, this material was tested clinically in 6 patients. In 3 of these, sufficient metabolic data have been obtained to date to permit charting, as shown in Fig. 1. The fourth patient (after this chart went to press) made a standard metabolic response up to plus 16, on a daily dose of material containing 25 mg. of iodine. The data in the other 2 are incomplete.

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[3] Abelin, I., *Arch. f. exp. Path. u. Pharmakol.*, 1936, **181**, 250.

[4] Wormall, A., *J. Exp. Med.*, 1930, **51**, 295.

Case 1. E. T., No. 77797, a 57-year-old woman, had myxedema for 20 years. The basal metabolic rate level was minus 36, pulse 50-54, and weight 72.5 kg. The plasma cholesterol was 284 mg./100 cc. She received material containing 5 mg. of iodine daily for 4 doses, 10 mg. for 4 doses, 20 mg. for 3 doses, and 30 mg. for 2 doses. Definite metabolic response started with the 10 mg. dosage. The subsequent metabolic response was rapid and persisted for a week after medication was stopped. She lost almost 4 kilograms in weight and had a definite diuresis. Clinically she became normal.

Case 2. L. B.,† No. 8702, a 25-year-old female cretin, was first seen by Dr. Fritz Talbot in 1915. The basal metabolic rate was minus 42 and minus 36, and weight averaged 36 kg. Plasma cholesterol was 331 mg./100 cc. She received material containing 5 mg. of iodine daily for 17 days. Coincident with the metabolic rise, she lost 4.5 kg. in weight and she appeared brighter and more animated.

Case 3. M. C., No. 3620, a 59-year-old woman, with typical myxedema of 3 years' duration, had a basal metabolic rate level of minus 37, pulse 60, and weight 74.5 kg. Plasma cholesterol was 390 mg./100 cc. She received material containing 15 mg. of iodine daily for 18 days. Her metabolism rose to a level of minus 14 and she lost 6 kg. in weight. There was a slight but definite diuresis, and plasma cholesterol was reduced to 121 mg./100 cc. Clinically she approached normal appearance. An electroencephalogram, obtained by Dr. R. S. Schwab, before treatment showed an alpha rhythm of 7-7.5 per sec., and a reaction time of 0.4 sec. Both values are extremely slow. After treatment, they approached normal, the alpha rhythm being 9.3 per sec. and the reaction time 0.3-0.35 sec.

In general, the activity is distinctly less than that of thyroglobulin and more of the order of diiodothyronine reported by Abderhalden and Wertheimer,[5] when dosage is based on total iodine.

Conclusion. It is shown in 3 cases that a derivative of iodinated protein obtained entirely from extra-thyroidal sources exerts qualitatively the physiologic action of thyroid hormone in human myxedema.

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† This case is reported in full in Means, J. H., *The Thyroid and Its Diseases*, Philadelphia, J. B. Lippincott Co., 1937, page 274.

[5] Abderhalden, E., and Wertheimer, E., *Z. f. d. ges. exp. Med.*, 1929, **68**, 563.

9754

Presence of Spirochetes in the Urine and Prostatic Fluid of Patients with Relapsing Fever.

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So far as is known spirochaetes have never been demonstrated in the urine or prostatic fluid of patients with relapsing fever. A study has been based on patients suffering from the Chinese type of this disease.

Altogether 45 specimens of urine from 41 patients with *Spirochaeta recurrentis* in the peripheral blood have been studied. In obtaining specimens for examination, only clean glass jars were used and only the second portion of the urine was collected. In most cases, the external genitalia were cleaned with soap solution, water and finally with a dilute solution of bichloride of mercury just prior to the collection of a specimen. In all instances, the urine was examined soon after it had been collected. To facilitate the search for spirochaetes, 10 cc. of each specimen of urine were centrifuged at high speed (2,000-2,500 r.p.m.) for 20 minutes. Four drops of the sediment on 4 different slides were examined under the darkfield microscope before a urine specimen was declared negative. In the positive specimens, it was not necessary to examine more than one or 2 drops of the sediment to find the spirochaetes. The urinary sediment of 3 patients was instilled onto the conjunctivæ and oral mucosæ of 3 squirrels respectively and that of 3 other patients was inoculated intraperitoneally into 3 other squirrels to ascertain whether or not the spirochaetes present were viable and virulent.

In most instances the urine was also examined for its reaction, albumin, sugar, casts, and blood cells. The duration of illness and the age of patients were also noted in every case. All patients were males, and none of them had any obvious urethral disease or genital lesions.

The urine of 27 (65.9%) of the 41 patients studied showed spirochaetes morphologically indistinguishable from *Spirochaeta recurrentis* present in the patient's blood. Most of the spirochaetes found were dead or motionless, but occasionally living spirochaetes were also encountered. One of the 6 squirrels inoculated with urinary sediments developed relapsing fever 18 days later with numerous *Spirochaetæ recurrentis* in the blood. The presence or absence of blood cells and casts and the duration of illness did not appear to have any definite relation to the presence of spirochaetes in the urine, although it appears almost certain that whenever there is considerable hematuria during the febrile period, more spirochaetes are likely to be present in the urine than otherwise. In 2 patients many spirochaetes were found in the urine during the febrile period when the blood was positive, but none were found in the urine passed during the remission when the blood was already negative.

During the febrile period prior to specific treatment, 6 patients with relapsing fever were subjected to prostatic massage for 30 to 40 minutes, but only 2 of them yielded a few drops of prostatic fluid. These 2 patients were Chinese males in their thirties on the 4th and 5th day of the disease respectively and both were free from genito-urinary disease. Because of the small amount of the fluid available for study, the specimen obtained from one patient (on the 4th day of illness) was employed for direct examination under the darkfield microscope and that from the other patient was inoculated intraperitoneally into a splenectomized squirrel. Although the specimen subjected to direct darkfield examination showed no spirochaetes, the other specimen infected the animal with relapsing fever as evidenced by the occurrence 7 days later of fever and numerous *Spirochaetæ recurrentis* in its blood.

Summary. The urine of 27 (65.9%) of 41 patients with relapsing fever contained spirochaetes morphologically indistinguishable from *Spirochaeta recurrentis*. In most instances the organisms were motionless or dead. One of the 3 squirrels inoculated with urinary sediment developed relapsing fever, showing that viable and virulent *Spirochaetæ recurrentis* were present in the urine. Viable and virulent *Spirochaetæ recurrentis* were also recovered from the prostatic fluid of one patient.

9755

Influence of Allantoin on Growth of Chicken Epithelial Cells and Chondroblasts in Culture.

H. J. Chu. (Introduced by R. J. C. Hoeppli.)

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Robinson[1] reported that he had found allantoin in the secretion of maggots which are used in the treatment of chronic wounds. While allantoin may play a rôle in the therapeutic result of maggot treatment, Robinson himself pointed out that allantoin alone could not fully take the place of living maggots from the therapeutic standpoint. Recently Shipp and Hetherington[2] published the results of their study on the influence of allantoin on the growth of chicken fibroblasts in tissue culture. They found a slight but not significant stimulation by allantoin. In studying the literature regarding the effect of allantoin on chronic wounds, one gets the impression that the growth of the epithelial cells is especially stimulated. For this reason, it seemed of interest to study the influence of allantoin on the growth of epithelial cells in culture. Since pure cultures of chondroblasts (fibroblasts) were also available, the experimental series with epithelial cells was supplemented by another series on chondroblasts.

The cultures were epithelial cells from the retina and chondroblasts (fibroblasts) from the cartilage of a femoral epiphysis of a 12-day- and 9-day-old chicken embryo. The epithelial cells had been transferred 9 times and the chondroblasts 8 times before they were used for the experiment. Cultures were carried out by the cover-slip-hanging-drop method and kept for 4 days. A freshly prepared 1% allantoin solution in distilled water was used as stock solution from which concentrations of 1/200 and 1/300 were made with Tyrode's solution. All solutions were sterilized by filtration through a Seitz filter, heating being avoided. The experiment for each concentration was carried out 3 times with epithelial cells and chondroblasts.

Epithelial Cells. Well growing cultures (9th passage) were divided in halves, one half being used for the experiment and the other one as control. The experimental cultures were placed in one drop of chicken plasma plus one drop of a mixture of equal parts embryonic tissue juice (2nd centrifugate) and allantoin solution (either 1/100, 1/200, or 1/300). The respective controls were kept in one drop of chicken blood plasma plus one drop of a mixture of equal parts embryonic tissue juice (2nd centrifugate) and Tyrode's solution. As mentioned before, each experiment with one of the 3 concentrations of allantoin solutions was carried out 3 times with epithelial cultures of the 9th, 10th and 11th passages.

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[1] **Robinson, W., *J. Parasitol.*, 1935, 21, 354.**

[2] **Shipp, M. E., and Hetherington, D. C., Proc. Soc. Exp. Biol. and Med., 1936, 35, 180.**

Chondroblasts. Corresponding experiments were carried out with chondroblast (fibroblast) cultures of the 8th, 9th and 10th passages; for chondroblasts the fourth centrifugate of embryonic tissue juice was used.

The hanging-drop cultures were kept at 37°C. for 4 days and every day, outline drawings were made with the help of a projectoscope.

Epithelial Cells. A definite retardation of growth in the experimental cultures as compared with the controls was noticeable. This retardation was pronounced in the 3 cultures with allantoin 1/100 and was very slight in the cultures with allantoin 1/300.

Chondroblasts (Fibroblasts). All cultures which had received allantoin in solutions 1/100 and 1/200 showed a definite retardation of growth which was in proportion to the allantoin concentration. Cultures treated with allantoin 1/300 either were of the same size as the controls or only very slightly smaller. The present reaction towards allantoin of the chondroblast (fibroblast) material, therefore, was different from that of the fibroblasts used by Shipp and Hetherington. It must be remembered, however, that their technique and the source of the cell material were slightly different.

Summary. Pure cultures of chicken epithelial cells and pure cultures of chicken chondroblasts (fibroblasts) under the influence of allantoin in different concentrations showed a slight retardation of growth which was in proportion to the allantoin concentration. In higher dilutions of allantoin neither a retardation nor a stimulation of growth could be observed.

9756

Mechanism of Precipitin Reaction. II. Fractionation of Immunologically Pure Precipitin.

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We have reported previously[1] that the antibody of Type I Pneumococcus in horse or rabbit serum is separable into fractions with different immunological and physical properties and that the separation can be achieved by immunological or chemical methods. We have now found that the immunologically pure precipitin of Type I Pneumococcus is itself fractionable.

Twenty-five cc. of the antipolysaccharide precipitin solution from Type I anti-pneumococcus horse serum prepared according to our method[2] was fractionated with saturated ammonium sulphate. The scheme of fractionation and the amounts of protein obtained in each fraction are given in the diagram (Table I).

These fractions of protein were separately dissolved in water and dialyzed against pH = 5.5 acetate buffer (0.005 m) at ice-water temperature until free from sulphate. The precipitates were centrifuged and redissolved in 0.85% NaCl solution and found to be completely precipitable by the homologous polysaccharide. They represent, therefore, a fraction of the antibody with isoelectric point at pH = 5.5. The protein in the supernatant fluids was also completely precipitable by the homologous polysaccharide. The isoelectric points of these protein fractions were estimated by determination of the pH at which maximum precipitation occurred. When N/1000 HCl was added drop by drop to the supernatant fluids, precipitation of the precipitin occurred in 1 and 2 but not in 3 or 4. Therefore, the isoelectric points of the antibody in supernatant fluids 1 and 2 must be less than 5.5. When N/1000 NaOH was added, precipitation occurred in supernatant fluids 3 and 4 but not in 1 or 2. Furthermore, maximum precipitation was reached in supernatant fluid 3, before the pH of the solution was high enough to turn the color of phenol red pink, but in supernatant fluid 4 precipitation occurred at a pH red to phenol red, and definitely pink to phenolphthalein. Therefore, the isoelectric point of the precipitin in supernatant fluid 3 must lie between 5.5 and 7.6, while

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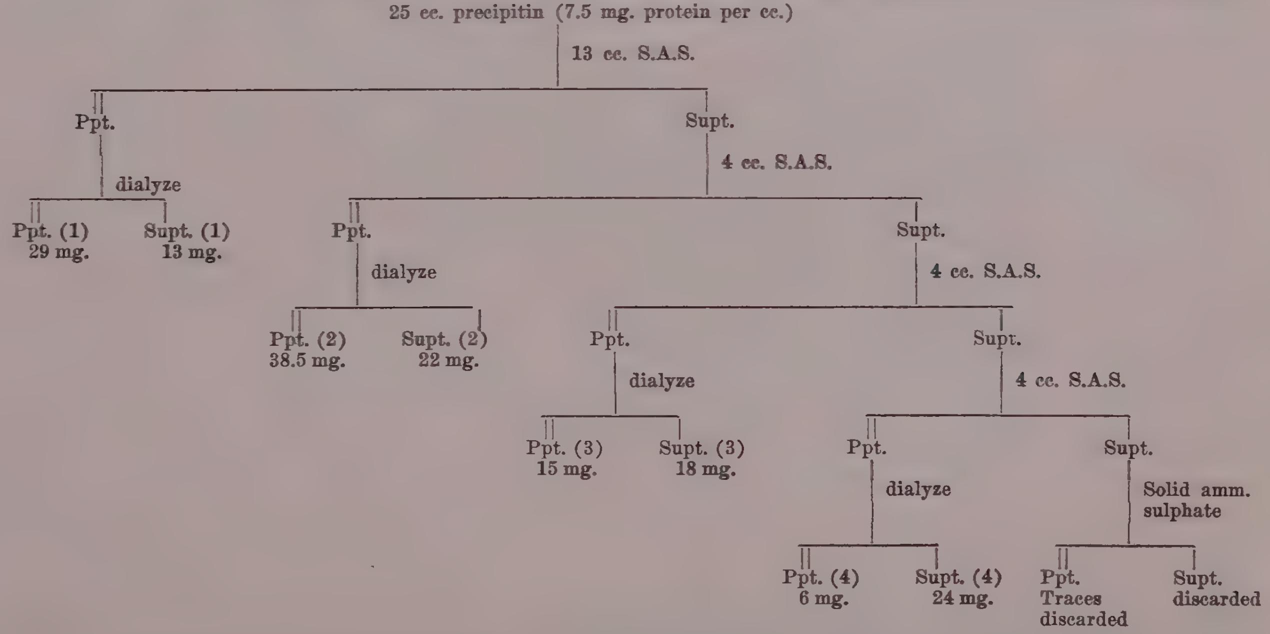
[1] Chow, B. F., and Wu, H., *Proc. of the Chinese Physiol. Soc.*, 1937, 18; Chow, B. F., and Wu, H., Proc. Soc. Exp. Biol. and Med., 1937, in press.

[2] Chow, B. F., and Wu, H., *Chin. J. Physiol.*, 1937, **11**, 139.

TABLE I.

Fractionation of Precipitin with Ammonium Sulphate.



S.A.S. = Saturated ammonium sulphate solution.

that in supernatant fluid 4 must be above 7.6. The original precipitin gave a maximum precipitation at pH 7.6 as previously reported.

These results show that ammonium sulphate can separate the precipitin into fractions with different isoelectric points. It will be recalled that the isoelectric point of the precipitin of Type I Pneumococcus prepared by Heidelberger and Kendall's[3] method of dissociation with a concentrated NaCl solution is 4.8, whereas that prepared by alkali treatment is 7.6. This difference may indicate that different fractions of the antibody are isolated by these two different methods.

9757

Hyperlipemia in Experimental Trypanosomiasis of Rabbits.

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Although changes in the lipoid content of the blood have been described in a variety of physiological and pathological conditions, they have not been sufficiently studied in animals with protozoal infections. Nakanishi[1] observed some increase in the serum cholesterol, fatty acids and phosphatides in the early stage of syphilis in rabbits but all these elements decreased when generalized infection was established. Santos[2] and Borel, *et al.*,[3] found that in patients suffering from malaria the serum cholesterol was subnormal. On the other hand, a slight increase in the blood cholesterol in filariasis was described by Boyd.[4] Linton[5] demonstrated some increase of plasma lipoid phosphorus in rats acutely infected with *Trypanosoma equiperdum*. In a study of anemia produced by the same organism Dubin[6] found in one dog a slight increase in total lipoid of whole blood and plasma accompanied by a decrease of cholesterol. Lannoy and Lagodsk[7] noted some elevation in the serum cholesterol of rabbits infected with *Trypanosoma annamense* without mentioning any variation in the total lipoid content. The changes so far recorded have not been constant and the variation from normal limits has been rather slight. In view of the meagre information available regarding the abnormalities of blood lipoids further investigation of the changes of these substances in the course of an experimental protozoal infection seems desirable. The present study was, therefore, undertaken to determine whether or not there is any change in the plasma lipoids of rabbits infected with *Trypanosoma brucei,* a strain which hitherto has not been used for the study of blood chemistry.

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[3] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1936, **64**, 161; Heidelberger, M., Pedersen, K. O., and Tiselius, A., *Nature*, 1936, **165**, 138.

[1] Nakanishi, M., *Jap. J. Derm. and Urol.*, 1936, **40**, 215.

[2] Santos, De Paula, *Trop. Dis. Bull.*, 1927, **9**, 377.

[3] Borel, Pons, Advier, and Guillerm, *Ann. Inst. Pasteur*, 1926, **40**, 152.

[4] Boyd, T. C., *Indian J. Med. Research*, 1930, **17**, 949.

[5] Linton, R. W., *J. Exp. Med.*, 1930, **52**, 103.

[6] Dubin, H., *J. Biol. Chem.*, 1913, **33**, 377.

Two series of rabbits were used, one consisting of 5 test animals, and the other, 4 controls. These animals were kept in separate cages and fed exclusively on 50 gm. of black beans per kilo weight plus a little cabbage. On account of the fact that during the later course of the infection the rabbits became sick and did not take as much food as the controls, the food of the latter was reduced by one-third in order to make it more or less comparable to the amount taken by the test animals. Body weight, temperature and blood count were determined once before inoculation and repeated at each bleeding. About 100,000,000 *Trypanosoma brucei* were injected intraperitoneally into the test animals on the day when the first bleeding for chemical determination was done. The establishment of the trypanosome infection in the test animals was proved by direct finding the organisms in peripheral blood in one case, and in 4 when they could not be so demonstrated by infecting white mice intraperitoneally with 0.5 cc. of whole blood. This suggests that invasion of the blood stream was usually not severe. Concurrent bacterial infection was ruled out by culturing the blood at short intervals. About 15 cc. fasting blood was obtained from ear-vein each time for chemical analysis. The total plasma lipoid, cholesterol, protein, non-protein nitrogen and sugar were determined. Bloor's titration method[8] was used for the estimation of total lipoid calculated in terms of oleic acid, Bloor's colorimetric method[9] for cholesterol and Howe's and Van Slyke's gasometric methods[10, 11] for protein. All chemical determinations were done in duplicate, and repeated when significant variations were encountered.

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[7] Lannoy, P. L., and Lagodsk, H., *Bull. Soc. Path. Exot.*, 1937, **30**, 57.

[8] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[9] Bloor, W. R., Pelkan, F. K., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 191.

[10] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 109.

[11] Van Slyke, D. D., *J. Biol. Chem.*, 1927, **71**, 235.

TABLE I.

	Plasma lipoid		Blood picture	
Time of Examination	Total fat, mg./100 cc.	Total cholesterol, mg./100 cc.	Hgb. grams	RBC mill.
Test animals				
Inoculation	152	50	10.6	5.79
2 weeks later	261	64	9.8	4.99
3 ” ”	702	163.5	7.7	4.55
*4 ” ”	5050	476	7.0	3.40
Inoculation	245	67	10.0	6.32
2 weeks later	309	54	8.0	5.02
3 ” ”	376	75.5	6.5	3.96
*4 ” ”	4500	370	7.0	4.01
Inoculation	324	72	10.0	7.35
2 weeks later	547	118	7.0	3.80
3 ” ”	670	185	6.4	4.64
4 ” ”	1188	182	7.5	4.09
†5 ” ”	1510	247	7.0	4.05
Inoculation	422	125	10.5	4.91
2 weeks later	551	154	9.0	4.37
3 ” ”	679	200	6.7	3.81
4 ” ”	1300	266	7.0	3.61
*5 ” ”	566	154	7.0	Not done
Inoculation	402	102	10.0	5.78
2 weeks later	435	88	9.0	4.37
*3 ” ”	688	133	6.3	3.81
Control animals				
Chemical analysis and blood count were done at the same times as those on the test animals.	149	40	11.2	
	196	57	11.0	
	187	51	10.0	
	225	40	10.0	
	168	32	11.0	
	227	57	11.1	
	182	34	9.6	
	152	47	10.0	
	249.5	61	11.5	
	331	93	10.2	
	161	36	9.8	
	152	34	10.5	
	166	44	10.0	
	144	42	11.5	
	177	42	10.8	
	200	32	8.5	
	191	38	10.5	
	122	20	10.0	
	105	19	9.8	
	200	38	9.0	

*Rabbits died few days after the last bleeding.
†Rabbit still living.

It is seen from Table I that spontaneous variation in the total lipoid and cholesterol content occurs in the blood plasma of rabbits of the control series. However, the change in fatty constituents in normal rabbits during 6 weeks' observation has never been significant. On the other hand, in the animals of the test series, there is a considerable increase both in the total lipoid and cholesterol content in 4 out of 5 animals. The maximal increases of total lipoid are 33, 18, 5 and 3 times the control figures respectively. Increases in the total cholesterol are also observed, namely 10, 6, 3 and 2 times respectively. It is interesting that normally the ratio of total lipoid to total cholesterol was 4 to 1, but after the infection was well established, in 3 animals, the ratio was changed to 12, 11 and 6 to 1. This disproportion of the total lipoid to the cholesterol clearly indicates that fatty constituents of the blood plasma, other than cholesterol, were also markedly increased. In one of the test animals which did not survive long enough to permit further investigation, the increase of the total lipoid was nevertheless significant. It may be mentioned that the plasma protein and sugar of the infected animals remained more or less within normal limits although the lipoid content was greatly increased. The explanation of these metabolic disturbances, however, is unknown at present.

Increased blood destruction induced by venesection and phenylhydrazine poisoning caused an increase of blood lipoids in rabbits.[12] However, the increase was not constant and was not significant unless the red blood cells and hemoglobin had declined below 50% of normal. In the present cases the anemia of the test animals produced by infection in addition to frequent bleeding undoubtedly played some contributory rôle toward the production of hyperlipemia and hypercholesterolemia, yet the increase in total lipoid and cholesterol was extremely marked in spite of the fact that the anemia was not severe. Moreover, the lipoid content was rapidly and continuously increasing while the anemia remained stationary.

Besides the data presented, it may be briefly stated that guinea pigs subjected to an overwhelming trypanosome infection with the same strain and surviving for 3 to 4 weeks, did not show any appreciable variation in their plasma lipoids.

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[12] Bloor, W. R., *J. Biol. Chem.*, 1920, **45**, 171.

9758

Immunological Studies of Polysaccharides of Encapsulated Bacillus. I. Isolation from Smooth *B. rhinoscleromatis*.

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It has been conclusively proved that the specific polysaccharide of pneumococcus possesses antigenic power. It is generally thought that similar substances obtained from other bacteria were devoid of any antigenic property. In the present study it will be shown that the substance prepared from *Bacillus rhinoscleromatis* is antigenic.

The organism was isolated at the Peiping Union Medical College Hospital from the infected submaxillary lymph gland of a clinically established case of rhinoscleroma infection. The cultural characteristics and biochemical reactions of this organism are similar to those described by Morris and Julianelle[1] for 10 strains of *Klebsiella rhinoscleromatis*. The organisms were grown on meat infusion agar, pH 7.6, for 48 hours at 37°C. after which they were washed with distilled water and precipitated by the addition of 95% alcohol. After standing in the ice chest over night the organisms were dried over a boiling water bath and then resuspended in distilled water in the proportion of one part of dried bacilli to 150 parts of water. The flask containing the organisms was thoroughly shaken and placed in the ice chest overnight. At the end of that time glacial acetic acid was added to the gelatinous suspension to effect a concentration of one percent. The flask was placed in a boiling water bath for 30 minutes. It is interesting to note that there was a clearing of the suspension after 15 minutes of heating as all the cells had settled to the bottom of the flask. At this point the flask was agitated and hydrolysis continued. After being allowed to cool, the clear yellowish solution obtained from centrifugation was precipitated with 4 volumes of 95% alcohol to which was added one percent sodium acetate. A fine, white, granular precipitate was observed. Upon standing for several hours the precipitate was collected by centrifugation and dissolved in a small volume of distilled water, centrifuged to remove insoluble substance and reprecipitated with 95% alcohol. Such a procedure was repeated until a perfectly water soluble substance was obtained, involving about 6 precipitations. It might be stated that a second treatment of the remaining intact cells with 1% acetic acid yielded practically no substance of the nature described above. The polysaccharide obtained will be designated as Fraction SI.

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[1] Morris, M. C., and Julianelle, L. A., *J. Inf. Dis.*, 1934, **55**, 150.

The remaining organisms were resuspended in distilled water and 10% potassium hydroxide solution was added to effect a concentration of 0.5%. After a few minutes in the boiling water bath there was a clearing of the suspension indicating complete cell solution. Heating was carried out for half an hour and after acidifying the solution with acetic acid to remove nucleoprotein, it was centrifuged and clarified through a Seitz filter. The solution was treated in the same manner as that described above. The polysaccharide appeared as a flocculent, gelatinous substance in contrast to the finely granular substance of the portion prepared by acetic acid hydrolysis. This polysaccharide will be designated as Fraction SII. A third polysaccharide was prepared from whole organisms by means of the alkaline method and will be designated as Fraction SIII. This last fraction was included for comparative purposes, since this is a common method employed by other workers in the preparation of bacterial polysaccharide of the Friedlander group.

Immune serum against the whole organism was prepared by the inoculation of rabbits intravenously with a heat-killed culture on 3 successive days followed by 4 days of rest; the whole course of injections was repeated twice making a total of 9 injections. Animals were bled 7 days after the last injection. If the titre of the serum was not sufficiently high as measured by the precipitation test with the polysaccharide a few more injections were given.

The 3 fractions obtained by the methods described above were light colored powders easily soluble in saline solution in the concentration of 0.1%; all contained varying amounts of nitrogen although the usual protein tests were negative with 1% solutions, gave a strong Molisch test, did not reduce Fehling's solution except after prolonged acid hydrolysis. Serologically they were indistinguishable at dilutions between 4,000,000 and 6,000,000, all gave precipitation with immune serum, and, at dilution 1:32,000,000, complement fixation with immune serum diluted 1-10.

By the same method employed for preparing immune serum against whole organisms rabbits were immunized with 5 and 10 mg respectively of the two polysaccharides in order to study their antigenic properties. Five days after the last injection the animals were bled and the sera were tested for complement fixation antibodies. The results are summarized in Table I.

It might be stated that the precipitation test was uniformly nega-

TABLE I.
Complement Fixation Reactions Between Immune Polysaccharide Sera and Fractions SI and SII.

Serum	Total mg. used for immuniz.	Test polys. fraction	Dilution of polysaccharide				
			1:1,000	1:5,000	1:10,000	1:50,000	1:100,000
S I	10	SI	4+	4+	4+	2+	—
SII	10	SI	3+	—	—	—	—
S I	10	SII	4+	3	—	—	—
SII	10	SII	4+	—	—	—	—

All sera diluted 1:5 in the test.

tive. For simplicity the results obtained with 5 mg. were omitted in the table since they were identical with those of 10 mg. From the table it is quite evident that Fraction SI is weakly antigenic while Fraction SII is insignificant in this respect. Furthermore its action is quite specific as shown by the fact that the titre for the homologous antigen is 10 times higher than that of the heterologous one. Complement-fixation with a suspension of the smooth organism served to substantiate the presence of antibodies. Positive result by complement fixation with anti-Fraction SI serum diluted 1:5 was obtained with a 1:200 dilution of a 24-hour-old agar slant culture (killed at 56°C. for one hour), while only the 1:10 dilution of the same suspension of the organisms showed a positive reaction with the anti-Fraction SII serum. A second series of injections did not improve the titre of the above sera. As will be shown in another paper of this series[2] Fraction SI prepared by acid hydrolysis is sensitizing while Fraction SIII prepared by alkaline hydrolysis is not.

The effect of alkali on the antigenicity of Fraction SI prepared by acid hydrolysis was studied in the following experiment: Part of the polysaccharide was treated with a half percent potassium hydroxide solution and boiled for 30 minutes. Fraction SI so treated with alkali was recovered by 3 precipitations in 95% alcohol. Rabbits were immunized with 10 mg. of this substance in the same manner as that described above. No demonstrable complement fixing antibodies in the sera of rabbits so immunized were found. This finding suggests that Fraction SI is the parent polysaccharide from which Fraction SII might be derived. If it were possible to convert the non-antigenic Fraction SII into the antigenic Fraction SI by boiling in acetic acid such a contention might be substantiated. Some data indicating that such is a possibility were obtained. Experiments along this line are in progress.

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[2] Kurotchkin, T. J., and Wong, S. C., in press.

Some of the properties of the polysaccharides may be summarized in Table II.

TABLE II.

Chemical and Serological Properties of Polysaccharide Fractions SI, SII and SIII.

Polys. fract.	% nitrogen	Ppt. titre	Complement fixation titre	Molisch test	Immunological properties
SI	1.5	1:4,000,000	1:32,000,000	Positive	Antigenic sensitizing
SII and SIII	1.5	1:4,000,000	1:32,000,000	"	Negative

Demonstration of the antigenic power of polysaccharides in rabbits by means of the complement fixation reaction has been recorded by Chow[3] for a polysaccharide prepared from Pneumococcus Type I and by Nishimura[4] for inulin, soluble starch and dextrine; the precipitation test was negative. In this paper we have shown that the same may be true of one of the polysaccharide fractions of *B. rhinoscleromatis.* The result of this experiment suggests that the method employed for isolation of the polysaccharides may be of importance in determining its antigenicity.

9759

Immunological Studies of Polysaccharides of Encapsulated Bacillus. II. Isolation from Rough *B. rhinoscleromatis.*

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It has been claimed by Julianelle[1] that the R form of Friedlander bacilli does not elaborate any soluble specific polysaccharide and as a result of this fact the organism is only species specific. It appears of interest to us to extend similar investigation to other closely related species of this group with the object of analyzing the antigenic structure of these strains.

Dissociation of the parent strain, *Bacillus rhinoscleromatis*, was carried out according to the method of Julianelle.[1] Rough variants began to appear as small non mucoid colonies on the eleventh daily transfer on 10% anti-S serum in meat infusion broth. We have found, however, that ageing the culture in 5% immune serum in meat infusion broth, pH 7.6, was equally effective in dissociating our strain, except that dissociants began to appear as early as the seventh day of incubation. Biochemical reactions of the dissociated culture were similar to those of the parent strain except that the production of acid in saccharose and rhamnose was delayed. The culture thus obtained fulfilled all the requirements of a rough strain. Briefly stated, the variant strain was agglutinated by anti-S serum to a titre of 1:512 while the parent encapsulated organism was agglutinated only to a titre of 1:16, it was also agglutinated by the homologous anti-R serum to a titre of 1:1024. By the India ink method, the organisms were found to be without capsules, and their virulence diminished; the parent strain itself was one of low pathogenicity for mice.

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[3] Chow, B., *Chinese J. Physiol.*, 1937, **11**, 223.

[4] Nishimura, S., *J. Exp. Med.*, 1929, **50**, 419.

[1] Julianelle, L. A., *J. Exp. Med.*, 1926, **44**, 683.

The rough culture used for the isolation of the polysaccharides was one which had been transferred daily on meat infusion agar, pH 7.6, for a period of 70 days. The methods employed for preparing the polysaccharides were the same as those reported previously.[2] The following designations will be used for the polysaccharides prepared: (a) Fraction RI, obtained by acid hydrolysis, (b) Fraction RII, obtained by alkaline hydrolysis of the cells previously used for acid hydrolysis, and (c) Fraction RIII, obtained from alkaline hydrolysis of the whole organism.

Immune serum was prepared in the same manner as that previously described.[2]

All the 3 fractions obtained were light colored powders easily soluble in 1% concentration in saline solution. All the usual protein tests were negative; all contained varying amounts of nitrogen, failed to reduce Fehling's solution but did so after hydrolysis with strong mineral acid, and gave a strong Molisch test in a 1:1,000 dilution. Some of the important serological and chemical properties are presented in Table I.

TABLE I.

Properties of Polysaccharides from Rough Strain of *Bacillus rhinoscleromatis*.

Polys. fraction	% yield	% nitrogen	Ppt. titre with anti-R and anti-S sera	Complement fixation titre with anti-R and anti-S sera, diluted 1:10
R I	5.7	2.2	1:2,000,000	1:8,000,000
R II	2.6	3.6	1:10,000	1:100,000
RIII	6.5	2.8	1:500,000	1:4,000,000

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[2] Wong, Sam C., in press.

From the table it is apparent that 2 distinct polysaccharides differing in nitrogen content and serological activity are present in the rough organism. Fraction RI prepared by acid hydrolysis, representing the surface polysaccharide, possesses the highest activity, while Fraction RII prepared by alkaline hydrolysis of the remaining intact cells previously treated with acetic acid possesses the lowest activity in spite of the highest nitrogen content. A further differentiation of the 2 polysaccharides was obtained by anaphylactic reactions. Fraction RI elicited symptoms of severe but not fatal shock in guinea pigs sensitized with polysaccharide, Fraction SI, prepared by acid hydrolysis of the smooth organism, when 2 mg. of the substance was administered intravenously, while Fraction RII was inert in similar amount.

It might be stated that a further purification of Fraction RIII did not improve the serological activity of the substance. An explanation of this finding may be obtained from a quantitative determination of the polysaccharide content of the rough organism. The results shown in Column 1, Table 1, are the averages of 6 different determinations in 2 different lots based on the dried weight of organisms. In other words, about 70% of the total polysaccharide of the organism is composed of highly active substance which apparently dominates its immunological specificity. It is quite clear that the diminished serological activity of Fraction RIII as compared to Fraction RI prepared by acid hydrolysis may be attributed to a mixture of both active and less active substances.

Serologically, it is difficult to distinguish Fraction RI of the R strain from the polysaccharides prepared from the smooth strain[2] either with anti R or anti-S serum since all these 3 polysaccharides behave similarly. However, certain differences between Fraction RI and the Fractions SI, SII, and SIII prepared from the smooth organism may be found in immune serum prepared by means of a well washed suspension of the decapsulated organism obtained by acid hydrolysis. Such immune serum agglutinated the R culture to a titer of 1:32 and it gave a very insignificant titre with Fraction RI (see Table II). Against its homologous chemically decapsulated organism an agglutination titre of 1:256 was obtained and against the S culture a titre of 1:16 was found. On the other hand, the chemically decapsulated organism was not agglutinated by anti R serum. The serological activity of such immune serum with various polysaccharide preparations from the smooth and rough organisms is summarized in Table II.

The above findings seem to indicate that by treatment with acetic

TABLE II.
Serological Activity of Serum Prepared with Decapsulated Organism.

Polys. fraction	Ppt. titre	Complement-fixation titre (serum diluted 1:10)
RI	1:1,000	1:10,000
RII	1:5,000	1:50,000
RIII	1:10,000	1:100,000
SI, SII and SIII	1:2,000,000-1:4,000,000	1:16,000,000

acid the smooth organism was only partially decapsulated. Three facts are difficult to reconcile with this assumption: (a) The chemically decapsulated organism was agglutinated by anti-S serum to a titre of 1:256. (b) Further treatment of the decapsulated organism with acetic acid did not yield any specific substance. (c) Fraction RI prepared by acid hydrolysis of the rough organism reacts poorly with the serum prepared with decapsulated organism in contrast to the high activity with anti-S serum. The exact explanation of these findings is not possible at present. The possibility of Fraction RI being derived from the remains of the capsule can not entirely be ruled out, the available evidence shows, however, that the bacteria from which the substance was prepared possess no demonstrable capsule and, therefore, the substance must have been probably derived from the cell itself.

Summary. Two immunologically distinct polysaccharides have been obtained from a rough strain of *Bacillus rhinoscleromatis,* one serologically active and the other more or less inert. Their chemical and serological properties are described.

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Immunological Studies of Polysaccharides of Encapsulated Bacillus. III. Active Sensitization with Polysaccharide from *B. rhinoscleromatis.*

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Attempts to produce active sensitization of guinea pigs through the use of killed cultures of different Gram negative microörganisms, have been, so far, uniformly unsuccessful. Since the development of hypersensitivity in infections caused by certain Gram negative bacteria is well established, it appears inconceivable that these bacteria should be incapable of causing active sensitization under experimental conditions. The failure in this respect might be attributable either to insufficient dosage or to unsuitable preparations of the antigen. In the present work it will be shown that the specific polysaccharide derived from a Gram negative, capsulated strain of *B. rhinoscleromatis* may act as an efficient antigen in bringing about active sensitization of guinea pigs.

Two lots of the specific polysaccharide were used in this experiment. The first lot of the substance (Fraction SI[1]) was prepared by a 30 minutes' hydrolysis of a watery suspension of bacterial cells in the presence of one percent acetic acid which was followed by alcoholic precipitation. The second lot (Fraction SIII[1]) was obtained by a similar process; 0.5% potassium hydroxide was used instead of acetic acid. From chemical analysis it was found that both lots of the substance represent polysaccharide fractions of the bacterial cell showing identical properties and similar serological behavior when tested against rabbit sera prepared by immunization with killed cultures of *B. rhinoscleromatis*. The important difference between the 2 lots of the substance consisted in finding that the polysaccharide prepared by acid hydrolysis proved to be antigenic in rabbits while that obtained by alkaline hydrolysis showed either very feeble antigenicity or none at all.

Two series of young guinea pigs, weighing from 250 to 300 gm., were sensitized in the following manner: The first series received two intraperitoneal and one subcutaneous injection of Fraction SI (prepared by acid hydrolysis). The total dose of the polysaccharide given to each animal was equal to 20 mg. The injections were made at 5-day intervals and the test for hypersensitivity was performed 10 days after the last sensitizing injection. The second series of animals was sensitized in a precisely similar manner using Fraction SIII (obtained by alkaline hydrolysis). The dosage and time intervals were maintained the same as in the previous series of guinea pigs. Hypersensitivity in these animals was tested by intravenous injections of measured quantities of each type of the substance. The result of the experiment is given in Table I. It was found that all animals sensitized with Fraction SI and subsequently injected intravenously with 2 or 4 mg. of the homologous substance or of the heterologous substance, Fraction SIII, developed acute anaphylactic shock. The general picture of the shock corresponded to the classical manifestations of protein anaphylaxis. Within 3 to 5 minutes symptoms developed, the animals showing profound

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[1] Wong, Sam C., in press.

TABLE I.
Table Showing the Result of Sensitization of Guinea Pigs with Specific Polysaccharides of *B. rhinoscleromatis.*

No. of animals	Polysaccharide used for sensitization Prepared by	Polysaccharide used for shocking Prepared by	Shocking dose in mg.	Result
2	—	acid hydrolysis	2-4	No response—control normal animals
2	—	alkaline hydrolysis	2-4	No response—control normal animals
6	acid hydrolysis	acid hydrolysis	2-4	Anaphylactic shock, survived
3	,, ,,	alkaline hydrolysis	2-4	Anaphylactic shock, survived
3	alkaline hydrolysis	acid hydrolysis	2-4	No response
3	,, ,,	alkaline hydrolysis	2-4	,, ,,

respiratory distress, spasmodic cough, urination, defecation and occasional jumping. Mild convulsions developed rarely. All the symptoms lasted for about 20 minutes, after which the animals showed a more or less prolonged state of general prostration. No fatal outcome has been observed in any of the animals tested. In contrast to the above described findings, the animals sensitized by the injection of the polysaccharide Fraction SIII did not respond to the intravenous administration of either type of the substance. From this experiment it follows that only the polysaccharide prepared by acid hydrolysis of bacteria (Fraction SI) possessed both sensitizing and shocking properties, while the polysaccharide obtained by alkaline hydrolysis (Fraction SIII) exhibited only shocking power.

Since the shock produced in guinea pigs which were sensitized by Fraction SI showed certain minor deviations from the typical anaphylaxis, *e. g.*, its course was markedly prolonged and no fatality occurred, it was thought necessary to corroborate our *in vivo* findings by an *in vitro* experiment. Accordingly 6 young virgin guinea pigs were sensitized, some received one fraction, the rest the other fraction; by the use of either polysaccharide the uterine horns of these animals were tested for hypersensitivity by the Schulz-Dale method. It was found that the addition, in concentrations ranging from 1:10,000 to 1:50,000, of each type of polysaccharide to the bath containing the uterine horns from the animals sensitized by Fraction SI resulted in a very marked and prolonged contraction of the horns. On the other hand, an entirely negative result was obtained when a similar test was performed with the uterine horns taken from the animals sensitized with Fraction SIII prepared by alkaline hydrolysis. The results indicate that our findings obtained *in vivo* are in perfect agreement with those seen in an *in vitro* experiment.

Our experiment shows that certain immunological characteristics which with a few exceptions have been attributed only to protein antigens may also be demonstrated in some bacterial polysaccharides, at least this is true with the soluble substance derived from *B. rhinoscleromatis.* Through the use of properly prepared polysaccharides it was possible in our hands to induce the formation of antibodies in rabbits and to induce active sensitization in guinea pigs.

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Relationship Between Female Sex Hormone and Dewlap in the Rabbit.

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Frazier and Mu[1] noted that prolonged administration of an estrogenic substance, extracted with butyl alcohol from the urine of pregnant women, feminized male rabbits. Among the physiological and anatomical changes observed was the development of a ruff, a prominent fold of loose skin encircling the anterior part of the neck. In well-developed cases the skin was so redundant that it could easily be pulled over the nose of the animal. A similar condition in normal rabbits is known to breeders as dewlap.[2] In order to ascertain the significance of this formation of skin, we have examined the rabbits in our breeding colony for incidence of dewlap, and have also made some observations on adult male rabbits and on immature male and female rabbits treated with estrogenic substance. Another series of female rabbits was subjected to ovariectomy. This we did on the assumption that dewlap is related physiologically to the female sex hormone of the animal.

The rabbits of the breeding colony were examined on 3 occasions, 6 months apart, in the spring and fall. Both male and female animals and their young were included in this study. They were of different breeds with a variation in age from birth to 3 years. In the case of the adult females, the physiological status (resting, pregnant, or lactating) and the number of pregnancies were taken into account.

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[1] Frazier, C. N., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 997.

[2] *Guide Book and Standard*, Chicago, Ill., The Am. Rabbit and Cavy Breeders Assn., 1932-1933.

The dewlap is best observed in profile. The positive observations were classified in 3 grades depending upon the prominence of the fold of loose skin in front of the neck. A grade I fold may not be shown in a profile photograph, in which case it can be demonstrated only by grasping the skin between the fingers. Folds of grades II and III are easily seen in profile.

The observations on normal animals may be summarized under the following headings.

Sex. Table I shows that 53.6% of the 250 adult female rabbits had a dewlap, but that only 7% of the 200 adult males had the condition. Furthermore, in the male rabbits the fold of skin was usually very small, that is, no more than grade I. In the majority of instances, the females presented a dewlap of either grade II or III.

TABLE I.

Adult male rabbits*			Adult female rabbits*		
No. of animals	No. with dewlap	Dewlap %	No. of animals	No. with dewlap	Dewlap %
200	14	7.0	250	134	53.6

*Six or more months of age.

Age. There was not a single case of dewlap among 200 male and female animals under 3 months of age. Among 50 male and female animals between 3 and 6 months of age no case of dewlap occurred. In 189 adult females whose exact ages were known, a dewlap was present in 51% of 86 animals between 6 and 12 months of age, and in 61% of 103 animals more than 12 months of age.

Physiological Status. Our observations indicated no correlation between the incidence of dewlap and the number of pregnancies in the females, or the number of matings in the males. Neither pregnancy nor lactation was found to have any definite influence on the incidence of dewlap, although a few lactating does seemed to have a less prominent fold than when they were in the resting stage.

Breed. The main breeding lines showed no significant differences in the incidence of dewlap. However, these were not pure stocks. Most of the animals had the coat colors of the Dutch and Chinese albino breeds. A few were English, and others were of a gray colored breed.

Dewlap in Estrinized and Ovariectomized Rabbits. The experimental observations were made on groups of male animals receiving injections of estrogenic substance and on groups of ovariectomized adult females and their normal litter-mates. The effect of estrogenic substance on immature male and female animals was tested also. All injections of estrogenic substance were given daily, 6 times a week, by the subcutaneous route. The estrogenic substance was extracted from the urine of pregnant women by the same method described previously.[1, 3]

Dewlap developed in all of the 24 adult male rabbits in the course of from one to 3 months following the daily injection of 20-60 rat units[4] of estrogenic substance.

Two immature male rabbits, aged one month, were treated with the estrogenic substance starting with a dosage of 3 R.U. daily. This was doubled once, and again, in 3 and 8 weeks respectively, and finally increased to 18-30 R.U. a day. Both animals began to show the dewlap at a little over 7 months of age. It was quite prominent in one of the 2 rabbits while the animal was receiving 30 R.U. a day, but disappeared entirely after the dose of estrogenic substance was reduced to 15 R.U. a day. The dewlap did not return during a period of 3 months through which the treatment at this dosage was continued. This observation suggests that the response is not initiated by small amounts of the estrogenic substance in the circulating blood and can be sustained only when relatively large amounts of the substance are present.

A third male, aged 2 months, was treated with an initial dose of 15 R.U. a day. This dose was doubled once, and then again, in 3 and 9 weeks respectively. In spite of this large dose the animal, like the above 2, did not develop the dewlap until it had passed the seventh month of age.

One immature female was subjected to the same treatment as the first 2 immature males which were its littermates. This female, like the 3 males, did not begin to show the dewlap until it, too, had attained the age of 7 months.

Effect of Ovariectomy. Four sets of female rabbits, totaling 31, were ovariectomized at the age of 4-6 months. Both ovaries and fallopian tubes were removed together with the surrounding fatty tissue. Not a single case of dewlap was observed in a period of 4-6 months after the operation. Of 23 non-operated female litter-mates, 11, or 48%, developed a dewlap.

Ovariectomy not only prevented the development of a dewlap but also had a marked effect upon it after its full development. Three adult females, aged 10 months or over, with a grade III dewlap were ovariectomized. In all of the 3 animals the dewlap was re-

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[3] Frazier, C. N., Mu, J. W., and Hu, C. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 65.

[4] Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.

duced from grade III to grade I. The reduction was not appreciable in the first month following ovariectomy, but gradually and progressively took place in the next 2-2½ months, and then halted. One animal was observed as long as 8 months after the operation, while the other 2 were observed 4 months.

Summary and Conclusion. Judging from the evidence at hand we believe that the dewlap is normally a secondary sex character of the adult female rabbit, and that its presence is dependent upon the action of a substance (or substances) related primarily to the ovary. It may develop to a limited degree in the male rabbit in which case it is assumed that the female sex hormone is present in amounts sufficient to initiate and maintain the existence of the dewlap. The following observations support these conclusions: (1) A prominent dewlap was present in about 50% of the normal adult females and in only 7% of the adult males examined. When present in the male animal the fold of skin was usually so small that it could not be seen in profile. (2) The administration of a butyl alcohol extract of the urine of pregnant women induced a pronounced dewlap in all treated male animals. The developmental process was reversible, the fold of skin disappearing when the administration of estrogenic substance was reduced. (3) The dewlap failed to develop in animals ovariectomized before the age of sexual maturity, but did develop in normal female litter-mates with a frequency equal to that observed in the breeding colony. (4) When ovariectomy was performed after the appearance of the dewlap, it gradually underwent almost complete involution.

It is apparent that the female sex hormone did not exert its influence directly upon the skin during the development of the dewlap. In the breeding colony, the animals normally showed no evidence of dewlap before they were 6 months old, the age at which the rabbit reaches adult status. In spite of the daily administration of estrogenic substance, even in large amounts, normal immature male and female rabbits did not respond with a dewlap until they had reached the age of 6 months. It would seem, therefore, that the action of the female sex hormone depends upon an intermediary to stimulate the growth of the dewlap, and that this intermediary factor is present only in the adult animal.

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Inactivation of Complement by Iodine.

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Complement may be partially or completely inactivated by a number of chemical reagents.[1-4] However, practically nothing is known about the nature of reaction which takes place between them. In this paper, we shall report a quantitative chemical reaction between complement and an oxidizing agent, namely, iodine.

The procedure of a typical experiment for the inactivation of complement in serum by iodine is as follows: A suitable amount (1.0 to 3.0 cc.) of freshly prepared guinea pig serum was pipetted into a series of eight 10 cc. volumetric flasks, to which varying amounts of 0.0100 N iodine solution were subsequently added. After standing at room temperature for 5 minutes, they were made up to volume and the units of complement were determined by the standard method. The results given in Table I show a proportionality between units of complement inactivated and the amount of iodine used except in flasks 2 and 3 in which the number of units of complement destroyed is small and the usual method of determination is not sufficiently sensitive to detect the difference. We have also determined the amount of iodine necessary for the complete inactivation of complement in a number of guinea pigs' sera, and found that the ratio of complement to iodine (C) is also constant as shown in Table II.

TABLE I.
Comparison of Degree of Inactivation with Amount of Iodine Added.

Flask No.	I_2 N/100 added (B) cc.	Total units of complement found	Units of complement destroyed (A)	$\frac{A}{B} = C$
1	0.0	100	0	—
2	0.1	92	8	80
3	0.2	88	12	60
4	0.4	59	41	102
5	0.6	40	60	100
6	0.8	24	76	95
7	1.0	5	95	95
8	1.1	0	100	—

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[1] Gordon, J., Whitehead, H. R., and Wormall, A., *Biochem. J.*, 1926, **20**, 1028, 1036, 1044.

[2] Osborn, T. W. B., *Biochem. J.*, 1933, **27**, 1425.

[3] Gordon, J., and Thomson, F. C., *Brit. J. Exp. Path.*, 1933, **10**, 249.

[4] Wadsworth, A. D., *J. Immunol.*, 1937, **33**, 297.

TABLE II.

Ratio of Units of Complement Inactivated to Iodine Used.

A. Guinea pig serum				B. Rat serum				C. Hog serum			
Vol. serum used cc.	Total units of complement found A	Iodine N/100 used B cc.	$\frac{A}{B} = C$	Vol. serum used cc.	Total units of complement found A	Iodine N/100 used B cc.	$\frac{A}{B} = C$	Vol. serum used cc.	Total units of complement found A	Iodine N/100 used B cc.	$\frac{A}{B} = C$
2	200	1.8	110	1	30	1.4	22	3	90	4.8	18
2	170	1.6	105	1	20	0.9	22	3	90	5.6	16
2	200	1.8	110	2	66	2.5	26	1	20	1.2	17
2	240	2.3	105	2	76	2.7	28	1	15	1.2	13
2	240	2.4	100	2	50	2.4	21	3	84	5.2	16
2	200	2.0	100	2	32	1.5	22	2	41	2.9	14
2	150	1.5	100	2	54	2.7	20	1	36	1.8	20
2	172	1.6	107	2	76	2.9	26	3	75	5.0	15
2	164	1.6	102	2	66	2.4	28	2	60	4.6	13
2	200	1.9	105	2	74	2.8	26	3	78	4.5	17
2	266	2.6	102	2	66	2.6	25	1	32	1.8	18
2	280	2.5	112	2	66	3.0	22	1	28	2.0	14
3	300	2.8	106	2	74	3.7	20	1	35	2.2	16
2	290	2.8	105	2	50	1.9	27	1	37	2.4	16
3	300	2.9	103	3	90	3.5	26	1	39	2.6	15
2	240	2.4	100	2	76	2.9	26	1	28	1.8	15
2	228	2.3	100	2	76	3.6	21	1	30	2.2	14
1	80	0.8	100	2	66	3.0	22	1	32	2.6	13
				2	66	2.3	29	1	14	1.0	14
				2	80	3.0	27	1	15	1.1	14
				3	100	4.3	23				
				3	100	4.3	23				
				2	80	3.8	21				
				3	100	3.8	26				
Mean			104				24				15

The complement in normal serum of other species of animals is also inactivated by iodine. The animals studied include rat, hog, duck, man, dog, and chicken. The value of the ratio of complement to iodine for rat and hog have been found to be also constant (Table II).

Gordon, Whitehead and Wormall[1] reported that inorganic salts can inactivate complement. In order to show that the inactivation by iodine is not due to a specific effect of iodine or iodide, the inactivated serum was dialyzed to remove iodide and any remaining iodine. The serum so treated was found to possess no alexic activity. Therefore, the inactivation of complement by iodine must be a chemical reaction, most probably an oxidation. In fact, the results just reported indicate that there is a stoichiometric relationship between the amount of iodine consumed and the amount of complement inactivated. This indicates that the inactivation of complement proceeds so rapidly that the first portion of iodine added is almost completely reduced by complement and only the excess of the reagent is reduced by other substances in serum.

Our finding suggests a simple and rapid method of determining the amount of complement in guinea pig serum. The actual method and its significance will be reported in another communication.

Conclusions. 1. Complement in sera of different species of animal can be inactivated by iodine. 2. The amount of iodine necessary to inactivate a definite amount of complement in the serum of a species of animal, is a constant. 3. That constant varies in different species of animal.

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Effects of Chronic Injection of Rats with Extract of Pituitaries of the Same Species.

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Until about a year ago studies dealing with the production of loss of sensitivity to anterior pituitary extracts in animals subjected to chronic administration of these extracts had quite uniformly shown that this phenomenon apparently was one of species specificity. It appeared that the sera of such animals exerted a protective action in normal animals only against the active substances of the pituitaries of the species used for the chronic treatment.* These findings have recently been supported by the work of Brandt and Goldhammer,[1] Sulman,[2] and Zondek and Sulman.[3]

Within the past year, however, data have accumulated which do not support such specificity. Parkes and Rowlands[4, 5, 6] have reported that the serum of rabbits which have become refractory to the gonadotropic principles of the pituitary of the ox inhibited in other rabbits the gonad stimulating substances of the pituitaries of sheep, horse and the human, of the urine of pregnant women and of pregnant mares' serum. This antiserum as well as the antiserum to extracts of horse-pituitary inhibited in immature rats the action of extracts of human hypophyses. In this test animal, however, these antisera failed to inhibit the gonad stimulating principles of the pituitaries of the sheep and pig. Further, it was found that such antisera produced effects on the reproductive system of normal rabbits and rats[4, 6, 7] and the thyroids of normal guinea pigs[8] resembling those obtained by hypophysectomy. This is in line with earlier findings which indicate that the chronic administration of heterologous hypophyseal factors produces a neutralization of the animals' own pituitary hormones.

That the refractoriness to pituitary extracts may not be limited by species specificity is also indicated by work of Thompson and Cushing.[7, 8] These workers found that antisera to the gonadotropic and thyrotropic principles of sheep's pituitaries inhibited the corresponding active constituents of the pituitaries of other species and of certain types of human urine. The work of Gregerson, Clark and Kurzrok[9] also conforms with these findings.

Zondek and Sulman[3] maintain that the production of gonadotropic inhibitory substances manifests strict species specificity. By means of quantitative determinations they showed that the inhibitory effectiveness of the antigonadotropic factors is very much less against a heterologous gonadotropic factor than against the homologous one. They point out that in qualitative determinations where excessive quantities of antiserum are used this difference may not be apparent. Parkes and Rowlands[4, 8] have also observed that some antisera are more effective against their own antigenic extracts.

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* For references see (14).

[1] Brandt, R., and Goldhammer, H., *Z. f. Immunitaetsforsch.*, 1936, **88**, 79.

[2] Sulman, F., *J. Exp. Med.*, 1937, **65**, 1.

[3] Zondek, B., and Sulman, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 712.

[4] Parkes, A. S., and Rowlands, I. W., *J. Physiol.*, 1936, **88**, 305.

[5] Rowlands, I. W., *J. Physiol.*, 1937, **90**, 19P.

[6] Rowlands, I. W., *Proc. Roy. Soc. London*, 1937, B**121**, 517.

[7] Thompson, K. W., and Cushing, H., *Proc. Roy. Soc. London*, 1937, B**121**, 501.

[8] Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 637.

[9] Gregerson, H. J., Clark, A. R., and Kurzrok, R., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 193.

In connection with the question of species specificity the production of a refractory state in animals receiving pituitary hormones of homozoic animals is of importance. Most attempts to produce inhibitory substances in this manner have failed. Positive results have been obtained by Selye, Collip and Thomson[10] with rats and Collip[11] with sheep. Thompson[12] injected sheep with sheep pituitary extract for a longer period than did Collip but failed to produce inhibitory substances. It is possible that the latter employed a more drastic procedure for the preparation of the extract which altered the antigenic properties of the active material.

In the present investigation we have attempted to produce a refractory condition in rats by the chronic administration of an extract of the pituitaries of the same species instead of implanting fresh anterior lobes as we had done previously.[14] This was done to determine whether an extract of the gonadotropic material of rats' hypophyses could be prepared which would react in a manner antigenically similar to active material of fresh anterior pituitary.

The anterior lobes, dissected from the posterior lobes of over 1000 rats, were desiccated by means of acetone. The dried glands were ground to a powder and weighed. Fresh extracts were prepared every 10-15 days by extracting a weighed portion of the powder with N/10 $NaHCO_3$, centrifuging and neutralizing the extract with N/10 HCl. Each rat was injected daily with an amount of extract equivalent to 2 anterior lobes.

The chronic administration of this extract was started on ten 55-60-day-old female rats. All animals remained in good health until they were sacrificed. At intervals between 107 and 255 days of treatment 2 of the animals were used in each test for refractoriness. Decapitation was performed under light chloroform anesthesia, the blood was combined and centrifuged and the serum used for the detection of gonadotropic inhibitory substances.

Table 1 contains the weights of some of the endocrine glands and the uteri (free of fluid) of these animals receiving the chronic

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[10] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

[11] Collip, J. B., *Can. Med. Assn. J.*, 1937, **36**, 199.

[12] Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 634.

[14] Katzman, P. A., Wade, N. J., and Doisy, E. A., *Endocrinol.*, 1937, **21**, 1.

TABLE I.
Effect of Pituitary (Rat) Extract on Adult Female Rats.

Injection period, days	Body wt. at autopsy, gm.	Ovaries, mg.	Uterus, mg.	Adrenals, mg.	Thyroid, mg.	Pituitary, mg.
107	172	568	580	37	16	11.1
107	168	228	726	34.5	10.5	9.2
133	205	613	705	50	18	10.3
133	198	286	440	31	20	16
184	215	155	—	47	22	13.6
184	208	200	—	44	16	10.2
241	210	215	546	51	16.5	9
241	245	320	516	60	21.5	17
255	269	190	1300	47	23	13
255	226	235	585	48	16.2	8
Aver.	212	301	675	45	18	11.7

treatment. The ovaries of 2 animals sacrificed after injection periods of 107 and 133 days respectively, were very much larger than those of the other animals. This probably was due to an unusual sensitivity since there was no progressive decrease in the weights of the ovaries of the other animals injected for longer periods. Even after 255 days of treatment the ovaries were still 400-500% larger than those of untreated controls. They were loaded with large corpora and follicles of various sizes which, histologically, appeared to be functional. The weights of the other endocrine glands did not differ significantly from those of untreated animals. The uteri, of course, were markedly enlarged.

The serum was tested for antigonadotropic activity in 21-day-old female rats by determining whether it would diminish the effect of the extract of rats' pituitaries. Each animal received a total of 2.1 cc. to 3.0 cc. of the serum administered subcutaneously twice daily on 3 successive days. The injection of the extract was begun one day after the first injection of serum. Each immature female received daily an amount of extract equivalent to 2 rats' pituitaries, administered in 2 subcutaneous injections for 3 successive days. The serum and extract were injected in widely separated sites to prevent any influence of the former on the absorption of the latter. Controls, receiving only the extract, were used for each test. All animals were killed 100 hours after the first injection of the extract, and the ovarian weights determined.

It is evident from Table II that in no case was there a significant inhibition of the response to the extract. In some instances we have observed an augmentation similar to that noted in our earlier work with fresh anterior lobe implants. It is interesting to note that this augmentation was manifested by the sera obtained from the rats which were treated for 107 to 133 days but not by the sera

TABLE II.
Influence of Serum of Chronically Treated Rats on Effect Produced by Injection of Pituitary (Rat) Extract in Immature Female Rats.

Treatment	No. of rats used	Body wt. (gm.)			Wt. of ovaries (mg.)		
		Min.	Max.	Aver.	Min.	Max.	Aver.
107-day serum + extract	4	39	43	41	36.5	46.3	41.8
Extract alone	4	35	44	41.8	20.3	31.3	23.7
133-day serum + extract	3	40	46	42.1	29.0	60.5	50.0
Extract alone	2	38.5	40	39.2	19.5	31.0	25.2
184-day serum + extract	5	40	46	42.6	14.9	55.7	35.5
Extract alone	5	36	43	39.2	16.8	38.7	29.6
241-day serum + extract	5	38	45	42	23.0	38.0	32.6*
Extract alone	5	32	37	34.8	31.0	46.0	36.9
255-day serum + extract	5	40	55	47.8	29.0	89.0	49.0
Extract alone	5	40	50	43.2	24.0	78.0	50.0

*An extract prepared from a different sample of powdered anterior lobes was used for this group and the succeeding groups of animals.

obtained from the animals treated for longer periods. Similar observations have been made by Collip[11] and Thompson.[13] We are at present studying the time of appearance and the nature of this augmentory substance.

It is unfortunate that a larger number of immature rats could not be used in this investigation, since the response to the extract was quite varied. However, owing to the large number of animals required to supply the hypophyses for the chronic treatment, the amount of such serum obtainable is small.

Summary. We have confirmed our previous findings that rats receiving chronic treatment with active principles of the anterior lobes of the same species fail to become refractory. Under the conditions of the experiment an aqueous extract of acetone-desiccated hypophyses obtained by extraction with dilute sodium bicarbonate produced effects similar to those of implants of fresh anterior lobes.

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[13] Thompson, K. W., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 640.

9764 P

Factors Limiting Bacterial Growth. II. Growth Without Lag in *Bacterium coli* Cultures.

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The continuation of studies already reported[1] has brought out two facts bearing on the question of bacterial lag. First, it has been found that from the moment of seeding and throughout the early hours of growth, the oxygen-uptake of cultures of *Bacterium coli* increases logarithmically, irrespective of the age of the organisms used for inoculation. Secondly, it was possible to show that the oxygen-consumption calculated per unit of bacterial nitrogen is constant during all phases of growth, when measured under uniform conditions.[2] From these observations, it was inferred that a plausible explanation of the phenomenon of lag was to be found in the changes in average size of cells during the early development of the culture. The experiment to be reported here served, therefore, both to confirm the manometric findings, and to provide direct evidence of the nature of the lag phase.

The data required were obtained by simultaneous determinations of bacterial numbers by plating, and of total bacterial nitrogen by micro-Kjeldahl analysis of the centrifugate,[3] at intervals throughout the growth-period. Beef-extract broth in 200 ml. amounts, containing M/100 phosphate buffer of pH 7.4, was seeded at 37°C. with aliquots of a 24-hour culture of *Bact. coli* grown in the same medium. The number of cells and the bacterial nitrogen represented by the inoculum were obtained by analysis of additional aliquots of the parent culture. At intervals, one of the seeded flasks was sampled for enumeration, and immediately killed either by addition of 0.5% formalin or, in other experiments, by heating at 60°C. for 10 minutes. Nitrogen was determined after centrifuging and washing the killed bacterial cells. Blank determinations were made by suspending small amounts of a bacterial suspension of known nitrogen-content in sterile broth, and recentrifuging. Typical results are shown in Fig. 1.

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[1] Hershey, A. D., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 556.

[2] In preparation.

[3] Mueller, J. H., *J. Bact.*, 1935, **29**, 383.

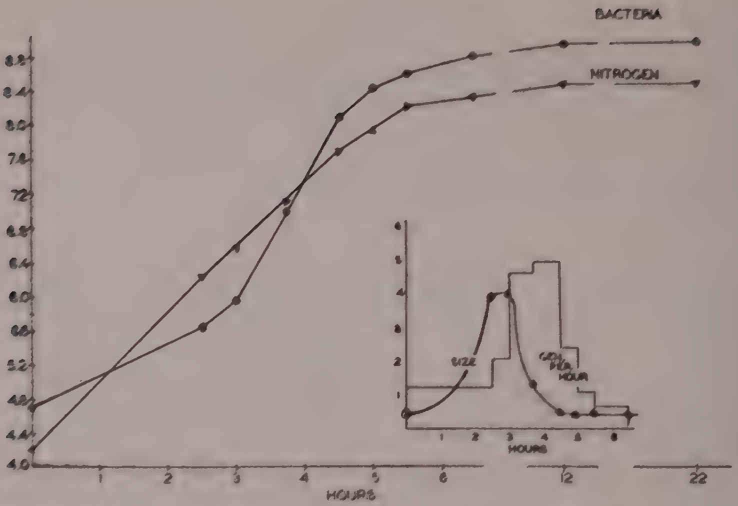

FIG. 1.

Increase of Population and of Bacterial Nitrogen in a Bacterium coli Culture.

The logarithms of numbers and of nitrogen in mg. $\times 10^{10}$ per ml. are plotted against time. The insert shows the changes in average size expressed as nitrogen in mg. $\times 10^{-16}$ per cell, and in the average rate of growth as reciprocal of generation time during the intervals observed.

It is clear that the rate of growth represented as increments of bacterial nitrogen is highest during the early hours of growth, and is already diminishing before the period of rapid multiplication has ended. The nitrogen-per-cell quotient shows a ten-fold increase during the initial period of retarded multiplication, and a corresponding decrease while the multiplication-rate is at its peak.

It is suggested that in view of these findings, the terms "phase of cell-enlargement" and "phase of rapid cell-division" better characterize the early events of growth under these conditions, than do the terms "phase of bacterial lag" and of "logarithmic increase."

The application of these facts to the problem of growth rates in artificial media is obvious. It is unnecessary any longer to postulate obscure physiologic states corresponding with the varying activity of *Bact. coli* at different times during the life of the culture. It is evident that mechanisms underlying the observed changes in the rate of growth are to be sought primarily in the analysis of environmental influences.

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Effect of Bacteriophage on Strepto-Fibrinolysin Production.*

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To test the effects of bacteriophage on the production of fibrinolysin by hemolytic streptococci, 100 cc. flasks of 0.5% glucose veal-infusion broth (pH 7.4) were inoculated with 0.1 cc. of a 24-hour broth culture of *Streptococcus hemolyticus* and an amount of bac-

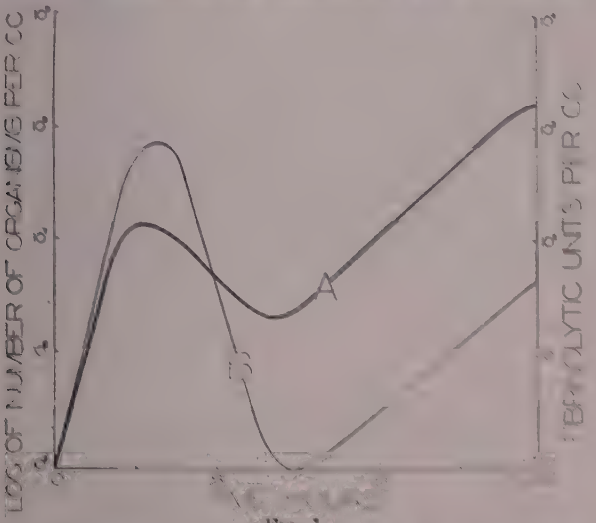

Fig. 1.

Phage-acceleration of Fibrinolysin Production.

100 cc. veal infusion broth, plus 1 cc. bacteriophage (homologous 'phage-lysed filtrate), inoculated with 0.1 cc. 24-hour broth culture of *S. hemolyticus*, incubated at 37°C with constant stirring.

A, fibrinolysin production. Composite data from 6 titrations with 2 different bacterium-phage combinations. For titration technic and definition of fibrinolytic unit see previous paper.[1]

B, parallel changes in total bacterial population as determined by the Petroff-Hauser counting chamber.

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† Eli Lilly and Co. Research Associate in Bacteriology, Stanford University, 1936-37. Park, Davis and Co. Research Associate in Immunology, Stanford University, 1937-........

[1] Madison, R. R., and Taranik, Jeannette D., Proc. Soc. Exp. Biol. and Med., 1937, 36, 1.

teriophage (phage lysed homologous streptococcus filtrate) demonstrably sufficient to produce lysis was added to each flask. The flasks were incubated at 37°C. with constant stirring and samples were withdrawn at regular intervals for total population counts and fibrinolysin titrations. Composite data from 6 such counts and titrations with 2 different streptococcus bacteriophage combinations are recorded in Fig. 1.

From this figure it is seen that the initial increases in fibrinolytic titer are roughly parallel with the logarithmic increase in bacterial population. Bacterial lysis is accompanied by a partial (and in some cases nearly complete) destruction (or binding) of the fibrinolytic enzyme. The beginning of a terminal overgrowth with phage resistant variants, however, is accompanied by a renewed and greatly accentuated formation of fibrinolysin. The fibrinolytic titer at the 24 hour stage of the terminal overgrowth is at least 10 times that of the maximum initial titer with the parent phage susceptible strain.

In order to confirm these results, phage resistant variants were isolated in pure culture from such overgrowths, and 0.1 cc. of a 24 hour sub-culture of each phage resistant variant was inoculated into 100 cc. flasks of veal infusion broth, control flasks being inoculated with an equal volume of a 24 hour broth culture of the homo-

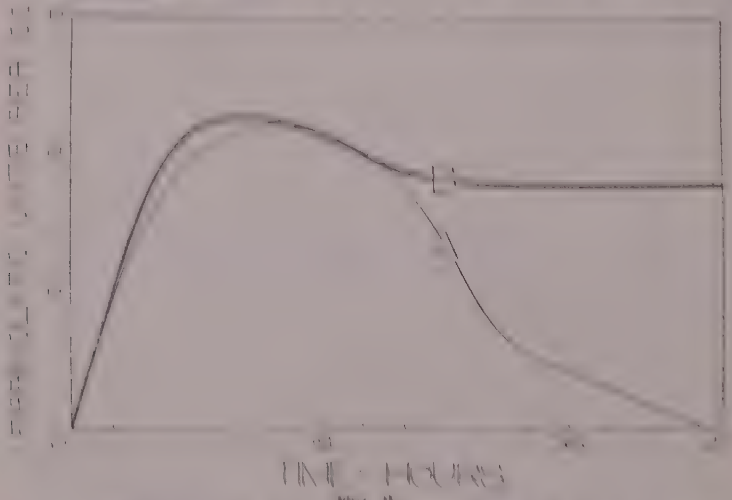

FIG. 2.

Fibrinolysin Production by Phage-resistant Variants

100 cc. veal infusion broth inoculated with 0.1 cc. 24 hour broth culture of *S. hemolyticus*, incubated 37°C. with constant stirring.

A, fibrinolysin production and subsequent destruction by control homologous phage-susceptible parent cultures. Composite data from 6 titrations.

B, fibrinolysin production by homologous phage-resistant variants. Composite data from 6 titrations.

logous, phage-susceptible parent strain. A typical comparison of fibrinolysin production by parent strain and phage-resistant variant is recorded in Fig. 2. Within the limits of the experimental error the population changes in both flasks were identical throughout the tests so are not recorded in the figure.

From Fig. 2 it is seen that while both flasks produced fibrinolysins at the same rate during the initial stages of population increase, the phage-susceptible parent strains rapidly destroyed or otherwise inactivated the fibrinolytic enzyme after reaching maximum titer. This destruction, however, did not take place (or was counterbalanced by continued fibrinolysin production) with the homologous 'phage-resistant variants.

If as a result of local phage injections all local streptococci could be destroyed, bacteriophage therapy would presumably aid in preventing the spread of streptococcus infections. The danger of the local production of phage-resistant variants with heightened fibrinolytic function and presumably heightened virulence,[2] however, would seem to contraindicate phage therapy in local streptococcus infections. This conclusion is in line with the experimental evidence reported by Bronfenbrenner and Sulkin[3] in their study of the local therapy in experimental staphylococcus infections in rabbits.

9766

Influence of Adrenalectomy and Choline on the Fat Content of Regenerating Liver During Fasting.

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Removal of the adrenal glands prevents the deposition of fat in the liver due to phosphorus poisoning,[1] that following the administration of anterior pituitary extracts,[2] that which generally accompanies total pancreatectomy,[3] and also interferes in other cases in which fat may wander from the fat depots to the liver.[4, 5] Choline prevents the fatty liver which occurs when a diet low in choline or in both choline and protein is fed[6, 7] but has no effect upon the fatty liver due to phosphorus poisoning[8, 9] or the other causes noted above,[9] with the possible exception of pancreatectomy.[10] The comparative influence of adrenalectomy and choline on the fat content of regenerating liver during fasting has been examined.

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[2] Fraser, F. H., and Madison, R. R., *Ibid.*, 1935, **33**, 307.

[3] Bronfenbrenner, J., and Sulkin, S. E., *Ibid.*, 1935, **32**, 1418.

[1] Verzár, F., and Laszt, L., *Biochem. Z.*, 1936, **288**, 356.

[2] MacKay, E. M., and Barnes, R. H., *Am. J. Physiol.*, 1937, **118**, 525.

[3] Long, C. N. H., and Lukens, F. D. W., *Am. J. Physiol.*, 1936, **116**, 96.

[4] Laszt, L., and Verzár, F., *Biochem. Z.*, 1936, **285**, 356.

[5] MacKay, E. M., *Am. J. Physiol.*, 1937, **120**, 361.

A very constant average of 65% of the liver [12] is easily removed from the albino rat.[11, 12] The remnant rapidly increases in size and if the rat is fasted becomes rather fatty, the fat content reaching a maximum in about 24 hours.[12] The experiments reported here were all terminated 24 hours after operation, the animals having fasted during this period but not before operation. Liver fat was measured by the saponification procedure.[13] When adrenalectomy was performed the glands were removed through the abdominal approach at the same time that the partial hepatectomy was carried out.

The results in Table I confirm the marked consistency in the amount of liver removed[12] by this method.[11] They show that more fat is deposited in the regenerating liver of fasting female than male rats (Table I). This is similar to the sex difference in the high liver fat levels reached in rats on a low choline and a low protein diet.[14] Adrenalectomy reduces the deposition of fat in the regenerating liver of fasting rats just as the removal of these glands interferes with fat transport to the liver in many other conditions.[1–5] Choline, on the other hand, has no influence. In almost every condition in which adrenalectomy prevents the transport of fat to the liver the administration of glucose, providing it is absorbed and may be oxidized, does likewise. In the present experiment also glucose gives the same effect as the removal of the adrenal glands in so far as liver fat is concerned. This point will be considered in more detail elsewhere.

Summary. During 24 hours of fasting after partial hepatectomy (65% of the liver being removed) in the albino rat the liver becomes very fatty. Higher fat levels are reached in female than in male rats. Adrenalectomy prevents a good part of this fat deposition in the liver. Choline is without effect.

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[6] Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1932, **75**, 405.

[7] Best, C. H., and Huntsman, M. E., *J. Physiol.*, 1935, **83**, 255.

[8] Best, C. H., MacLean, D. L., and Ridout, J. H., *J. Physiol.*, 1935, **83**, 275.

[9] MacKay, E. M., and Barnes, R. H., *Am. J. Physiol.*, in press.

[10] Best, C. H., Ferguson, G. C., and Hershey, J. M., *J. Physiol.*, 1933, **79**, 94.

[11] Brues, A. M., Drury, D. R., and Brues, M. C., *Arch. Path.*, 1936, **22**, 658.

[12] Raulston, B. O., *Arch. Path.*, in press.

[13] Leathes, J. B., and Raper, H. S., *The Fats*, London, Longmans, 1925.

[14] MacKay, E. M., *Am. J. Physiol.*, 1937, **119**, 783.

TABLE I.

Exp.	Grp.	Sex	No. Rats	Treatment	Body Wt., gm.	‡Body Surface, sq. cm.	Liver per 100 sq. cm. Body Surface		Liver Fat	
							Removed at operation, gm.	When killed 24 hr. later gm.	%	Mg. per 100 sq. cm. B.S.
1	1	♀	5	Control	182	365		.712	17.2	122
	2	♀	4	*Choline	179	360		.850	20.1	171
	3	♀	4	Adrenalectomy	167	344		.598	11.3	68
	4	♂	5	Control	187	371		.797	14.3	114
	5	♂	4	*Choline	189	374		.759	13.7	105
	6	♂	5	Adrenalectomy	190	375		.667	5.4	36
2	1	♀	4	Control	186	370	1.067	1.003	17.9	180
	2	♀	3	Adrenalectomy	176	356	1.078	.688	13.3	91
	3	♀	3	Control	160	334	1.072	1.090	20.7	226
	4	♀	3	Adrenalectomy	190	375	1.032	.689	9.0	62
4	1	♀	3	Control	194	380	1.027	.803	20.1	161
	2	♀	3	,,	186	370	1.049	.800	19.7	158
	3	♀	3	†Glucose	195	382	1.280	.739	8.7	64
	4	♀	3	† ,,	186	370	1.001	.686	12.5	86
	5	♀	3	Adrenalectomy	183	366	1.000	.597	11.3	68
	6	♀	3	,,	181	363	.996	.532	10.1	54
5	1	♂	5	Control	162	337	1.091	.748	14.8	111
	2	♂	5	*Choline	158	331	1.046	.789	13.9	110
	3	♂	5	Adrenalectomy	161	335	1.078	.612	4.1	25

*40 mg. per 100 sq. cm. body surface of Choline chloride (Merck) in 10% solution by stomach tube at time of partial hepatectomy and repeated 12 hours later.

†300 mg. per 100 sq. cm. body surface of glucose in 30% solution.

‡"Body Surface" calculated from formula of Carman and Mitchell (*Am. J. Physiol.*, 1926, **76**, 380).

9767 P

Effect of Ablation of Sensory Cortex upon the Threshold to Pain Reaction.*

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From the Department of Neurology and Neurological Surgery, College of Medicine, University of Illinois.

Head,[1] elaborating upon the work of Dejerine, located the sensory level for pain perception in the thalamus. Since then there has been accumulated a vast amount of clinical material stressing the importance of this sensory level for pain sensibility. It has also been shown that 2 other forms of sensory intensity discrimination are not permanently diminished by ablation of relevant sensory cortical areas. Lashley,[2] in rats, and Marquis and Hilgard,[3] in monkeys, found no significant loss of brightness discrimination after ablation of the occipital cortex. Ruch,[4] found no loss in weight discrimination after ablation of the sensory cortex in monkeys. The development of a quantitative, sensitive experiment capable of differentiating the rôle of cortex and thalamus in establishing the threshold to pain reaction was therefore considered desirable.

Adult cats, of over 5 pounds, were used. In 5 of these animals, one hind leg cortical sensory area, as delimited by Bard and Brooks,[5] was removed by their technic. Marshall, Woolsey and Bard[6] have shown by action currents that the tactile sensibility of the entire body, excluding the face, is represented contralaterally in the cortex. All of these 5 animals showed a permanent loss of the dorsal placing reaction of Bard only on the contralateral hind leg, without motor disturbances. The dorsal placing reactions were present on the 3 normal legs. In one animal, No. 8, the sensory and motor area of the right hind leg was destroyed, which gave permanent loss of the dorsal placing reaction on both right legs, and a transient extensor paralysis and walking defect on the right hind leg. In one animal, No. 12, the entire right cortex back of the motor area to the occipital

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* Aided by a grant from the Graduate School of the University of Illinois.

[1] Head, H., and Holmes, C., *Brain*, 1911, **34**, 107.

[2] Lashley, K. S., *J. Comp. Neur.*, 1926, **41**, 1.

[3] Marquis, D. C., and Hilgard, E. R., *Brain*, 1937, **60**, 1.

[4] Ruch, T. C., *Assn. for Research in Nerv. and Ment. Diseases Publications*, 1935, **15**, 289.

[5] Bard, Phillip, and Brooks, Chandler M., *Assn. for Research in Nerv. and Ment. Diseases Publications*, 1934, **13**, 107.

[6] Marshall, W. G., Woolsey, C. N., and Bard, Phillip, *Science*, 1937, **85**, 388.

cortex was electro-coagulated. Placing reactions were absent on both left legs, but present on both right legs.

Experiments were begun 3 weeks after the operation. The animals were placed in a box with a head holder which kept the head fixed, but allowed free observation of the eyes. After a variable period of training, lasting from 3 to 25 days, the animals would remain quiet throughout the experiment. Thus, no anesthesia was necessary. The middle toe pads were stimulated by unipolar electrodes, connected to an inductorium with the hammer silenced. All sources of conditioning were avoided. Readings were taken one minute apart, and the alternate thresholds required to elicit pain reaction from stimulation of both normal and cortically ablated hind legs were determined.

The pain reaction chosen as the indicator was the reflex dilation of the pupil. This dilation occurs solely by an inhibition of the constrictor tonus mediated through the Edinger-Westphal nucleus. Cutting the oculomotor nerve abolishes the reaction. Absence or presence of the cervical sympathetic does not alter the threshold of the reaction. Deep narcosis or atropine abolishes the reaction. As a control 2 cats were prepared with unilateral vago-sympathectomy. Both eyes responded with dilation at the same threshold. That this reaction is exclusively an inhibition was first clearly stated by Bechterew,[8] elaborated by Braunstein,[9] and reaffirmed by Bain, Irving and McSwiney.[10] Further, Ury and Gellhorn, in unpublished work, confirm these previous investigators.

The animals with cortical lesions were placed in the box, and the head illuminated by a strong, constant light. The eyes were observed through a low-power microscope, fitted with a graduated scale, on which one division equalled 1 mm. of pupillary diameter. A dilation of one division was considered subthreshold, and a dilation of 2 divisions as the threshold reaction. This reaction occurs within the second of stimulation, and is reversible in from one to 2 seconds. The pupillary reaction is the constant and unfailing precursor of more violent pain reactions, such as moving or crying. A shortening of the coil distance 0.5 cm. from the point which gave the threshold pupillary reaction gave these stronger, more generalized reactions. Both eyes react simultaneously and to the same degree, irrespective

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[7] Dusser, de Barenne, *Assn. for Research in Nerv. and Ment. Diseases Publications*, 1935, **15**, 265.

[8] Bechterew, W., *Pfluger's Arch.*, 1883, **31**, 60.

[9] Braunstein, *Zur Lehre von der Innervation der Pupillen Bewegung*, Wiesbaden, 1894.

[10] Bain, W. A., Irving, J. T., and McSwiney, B. A., *J. Physiol.*, 1935, **84**, 323.

of the leg stimulated. Under these experimental conditions the dilation of the pupil in response to an afferent stimulus is a constant, sensitive indicator of a central process associated with pain. It is caused by a central inhibition, and is uncomplicated by any variable factors in the periphery.

Results are best expressed by graphs. Twenty-six experiments were done on 5 animals which had unilateral ablation of one hind leg sensory zone. In 23 of these experiments no essential difference in threshold was noted between the normal and ablated side. In 2

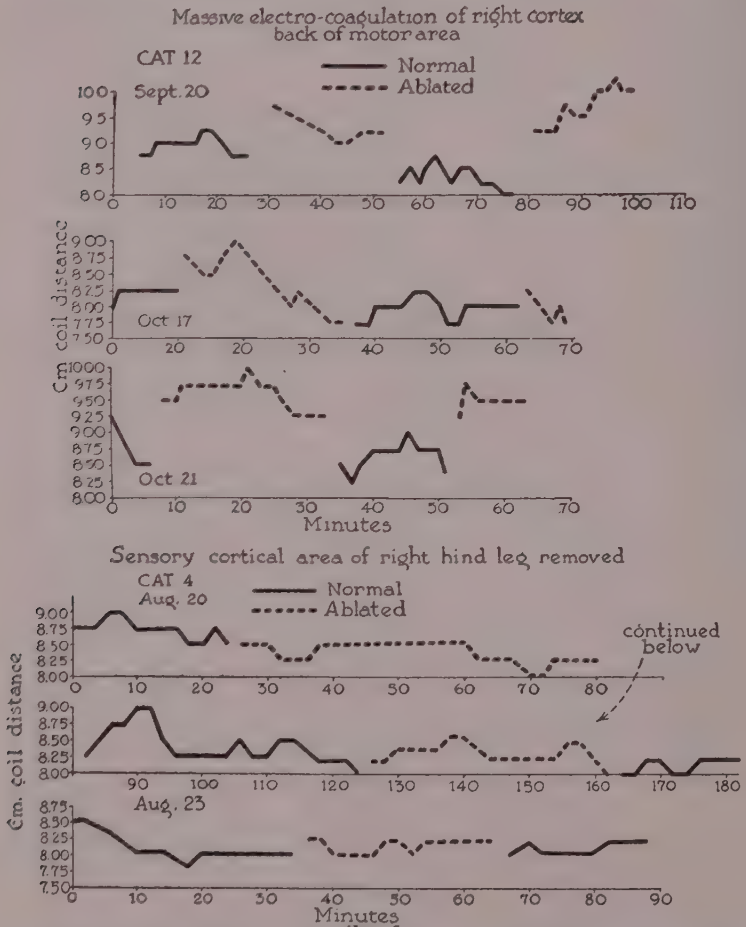

FIG. 1.

The graphs show the coil distance necessary to obtain the threshold reaction. The ordinate is coil distance in cm., the abscissa is time in minutes.

experiments the ablated side was slightly more sensitive, ½ cm. on the coil and in one experiment the normal side was slightly more sensitive. Twelve experiments were done on Animal No. 8, with sensori-motor ablation. In 3 experiments the normal side was slightly more sensitive, ½ cm. on coil, and in one experiment the ablated side was more sensitive to the same degree. Nine experiments were done on Cat No. 12, which had massive electro-coagulation of the right cortex. In 7 of these experiments the animal showed a greater sensitivity when the leg projected to the ablated side was stimulated. This increased sensitivity showed a gradual decrease during the 4 months this animal has been studied, during which time the normal side retained a comparatively constant level of response. Whether this is an individual response or a general tendency of such preparations is to be further investigated. Further experiments involving conditioned reflexes, frontal lobe ablations, and histological study of the brain have been initiated.

Conclusion. Ablation of the sensory cortex alone, or of the sensory and motor cortex, does not raise the threshold to pain reaction. In one animal massive unilateral ablation of the cortex made the animal more sensitive to painful stimuli applied to a body surface projected upon the ablated cortex.

The authors wish to thank the Physiology Department of the College of Medicine for the use of space and of apparatus essential to the performance of these experiments.

9768

Respiratory Quotient of Diabetic Liver.

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Studies have been published on the metabolism of the liver tissue excised from animals in various nutritive states; on normal diet, or during high fat feeding, as well as of fasted animals. Gemmill and Holmes[1] have reported the respiratory quotients of rats on well-balanced rations to average 0.78, and high fat diet, 0.58. Marsh[2] obtained similar results with fasted rats whose respiratory quotients average from 0.59 to 0.60. Elliott and Baker[3] noted a respiratory quotient as low as 0.46 in rats fasted for 20 hours. It is the purpose of this paper to extend these studies to include the respiratory quotient of the liver of the depancreatized animal.

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[1] Gemmill, C. L., and Holmes, E. G., *Biochem. J.*, 1935, **29**, 338.

[2] Marsh, M. E., *J. Nutrit.*, 1937, **13**, 109.

[3] Elliott, K. A. C., and Baker, Z., *Biochem. J.*, 1935, **29**, 2433.

Liver slices from cats depancreatized 48-72 hr. were studied in the Warburg respirometer. The respiratory metabolism was determined with the tissue suspended in saline solution buffered with phosphate at pH 7.4. Because of the reduced oxygen consumption of the fatty tissues it was found necessary to use 250 mg. instead of the usual 150 mg. Nevertheless, in some experiments the respiratory exchange was too small to be used to calculate the R.Q.

Thirty-nine significant observations yielded an average R.Q. of 0.59 ± 0.04; 5 observations fell within the range of 0.42-0.49; 27 were between 0.50 and 0.69 and 7 between 0.70 and 0.75. The average oxygen uptake of liver per 100 mg. tissue per hr. is 48 cu. mm., with values varying from 24 cu. mm. to 83 cu. mm. It should be mentioned that this average is much lower than that obtained from normal cats, which is 94 cu. mm. per 100 mg. tissue per hr. The diminution of oxygen consumption may be correlated with the unusually high fat content of the diabetic liver.

The determination of R.Q.'s of excised tissues in general yield results between the physiological values of 0.7 and 1.0. Of all the excised tissues examined, only the liver yields values consistently below 0.7 when fat constitutes the chief part of the foodstuffs oxidized by the animal. Since the R.Q. is an indicator, not only of the substrates oxidized but also of the intraconversion of foodstuffs, it is important to evaluate the significance of these low R.Q.'s. Gemmill and Holmes[1] observed rats on a high butter intake and noted that after the first day, during which time the glycogen content of the liver decreased to practically zero, there is a gradual increase of hepatic carbohydrate reaching the level of almost 1% on the fourth or fifth day of butter feeding. Taking these data in conjunction with the low R.Q., they concluded that fat was transformed to carbohydrate. Cori and Shine[4] were able to show that the glycerol fraction, which is 1/10 of the weight of fat, might be the substance used in the formation of this glycogen. In a more recent paper by Bach and Holmes[5] the increase of the glycogen content was also correlated with changes in protein metabolism, namely, the deaminization of amino acids and the subsequent conversion of the acid remainders to glycogen. Thus, it is apparent that the increased glycogen need not necessarily be imputed to the conversion of fat to carbohydrate.

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[4] Cori, C. F., and Shine, W., *Science*, 1935, **82**, 134.

[5] Bach, S. J., and Holmes, E. G., *Biochem. J.*, 1937, **31**, 89.

The low R.Q. may be ascribed chiefly to the incomplete combustion of fat. Both *in vivo*[6] and *in vitro*[7] it has been demonstrated that the liver is a source of acetone substances, products of incomplete combustion of fat. The complete combustion of fat yields a R.Q. of 0.7. However, the combustion to the 4-carbon stage would give a R.Q. below 0.7, since the acetone substances themselves possess R.Q.'s above that value. According to Knoops' classical theory of β-oxidation, the R.Q. of palmitic acid with the production of diacetic acid would be 0.63. However, with Jowett and Quastel's conception[8] of multiple alternate oxidation the R.Q. may fall to any level.

Another factor which must be taken into consideration for the production of low hepatic R.Q.'s is that of the function of the liver to produce urea, for the carbon utilized in this process would not be eliminated in respiration. This phenomenon may, therefore, lower the R.Q. Whatever the exact processes involved in the production of this low R.Q. may be, there is a striking correlation between the average values of the R.Q.'s of the liver of fat-fed (0.58), fasted (0.59-0.60), and depancreatized animals (0.59),

Summary. Thirty-seven observations of the R.Q. of the liver of cats depancreatized 48-72 hr. revealed an average R.Q. of 0.59.

9769

Gastric Vagi in the Dog. Erroneous Assumption of Uninterrupted Vagal Innervation in the Pavlov Pouch.*

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The fundamental difference in concept between the Pavlov pouch and the Heidenhain pouch is that the vagal innervation of the former is intact. Fig. 1, reproduced from Khigine[1] and Pavlov,[2, 3]

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[6] Goldfarb, W., and Himwich, H. E., *J. Biol. Chem.*, 1933, **101**, 441.

[7] Jowett, M., and Quastel, J. H., *Biochem. J.*, 1935, **29**, 2181.

[8] Jowett, M., and Quastel, J. H., *Biochem. J.*, 1935, **29**, 2159.

* Supported in part by a grant from The Friedsam Foundation.

[1] Khigine, P. P., *Arch. des Sciences Biol.*, 1894, **3**, 461.

[2] Pavlov, I. P., *Ergeb. d. Physiol.*, 1902, **1**, part 1, 246.

[3] Pavlov, I. P., *The Work of the Digestive Glands*, 2nd ed., London, 1910.

indicates the anatomical concept upon which they based their operative procedure. According to this diagram, after the anterior vagus trunk leaves the anterior gastric plexus, it descends the anterior surface of the stomach along the lesser curvature to the pylorus. The main posterior vagus trunk, however, after leaving the posterior gastric plexus, descends the corresponding stomach surface along the greater curvature to approximately the beginning of the pars pylorica. The incision which separates the pouch from the main stomach passes between these two main trunks and their arborizations. Consequently, the posterior trunk passes intact through the sero-muscular isthmus which joins the pouch to the stomach proper, insuring the integrity of the vagus supply to the pouch.

With only minor modifications in technique, the method of Khigine and Pavlov has been utilized by practically all physiologists who found use in their work for a "vagus-intact" pouch. These investigators, as well as other writers on the subject, state without reservation that in the Pavlov pouch the integrity of the nervous connections is preserved, and most of them employ as illustration the Pavlov diagram shown herewith. (Cannon,[4] Lönnquist,[5] Bickel and Katsch,[6] Thomsen,[7] Carlson,[8] Smidt,[9] Loeper,[10] Bayliss,[11] Boldyreff,[12] Lim, Ivy, and McCarthy,[13] Wright,[14] Babkin,[15] Starling,[16] Howell,[17] Bodansky,[18] MacLeod,[19] and Mathews.[20])

This classic description of the course of the posterior gastric

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[4] Cannon, W. B., *Am. J. Med. Sci.*, 1906, **131**, n.s., 563.

[5] Lönnquist, B., *Skan. Arch. f. Physiol.*, 1906, **18**, 194.

[6] Bickel, A., and Katsch, G., *Chirurgische Technik zur Normalen und Pathologischen Physiologie des Verdauungs Apparates*, Berlin, 1912.

[7] Thomsen, E., *Studier over Neurogen og Cellulaer Achyli*, Copenhagen, 1921.

[8] Carlson, A. J., *Phys. Rev.*, 1923, **3**, 1.

[9] Smidt, H., *Arch. f. Klin. Chir.*, 1923, **125**, 26.

[10] Loeper, M., *Histoire de la Secretion Gastrique*, Paris, 1924.

[11] Bayliss, Sir W. M., *Principles of General Physiology*, 4th éd., London, 1924.

[12] Boldyreff, W. N., *Ergeb. Physiol.*, 1925, **24**, 399.

[13] Lim, R. K. S., Ivy, A. C., and McCarthy, J. E., *Quart. J. Exp. Physiol.*, 1925, **15**, 13.

[14] Wright, S., *Applied Physiology*, 2nd ed., London, 1928.

[15] Babkin, B. P., *Die Aussere Sekretion der Verdauungsdrüsen*, 2nd ed., Berlin, 1928.

[16] Starling, E. H., *Principles of Human Physiology*, 5th ed., Phil., 1930.

[17] Howell, W. H., *A Textbook of Physiology*, 11th ed., Phil., 1930.

[18] Bodansky, M., *Introduction to Physiological Chemistry*, 3rd ed., New York, 1934.

[19] Macleod, J. J. R., *Physiology and Biochemistry in Modern Medicine*, 7th ed., St. Louis, 1935.

[20] Mathews, A. P., *Principles of Biochemistry*, New York, 1925.

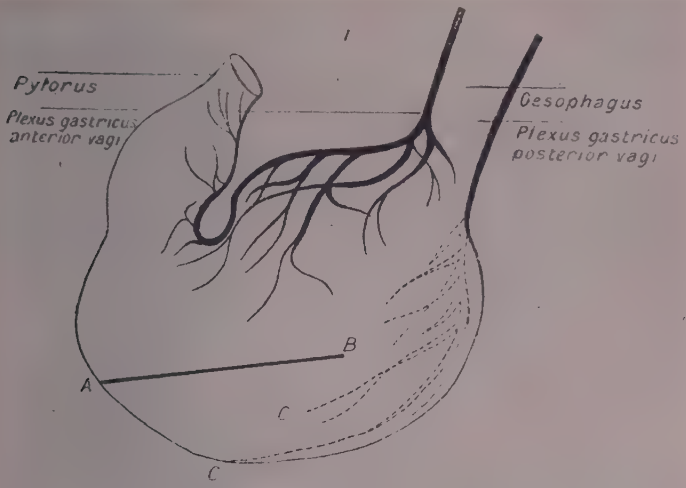
Pylorus
Plexus gastricus anterior vagi
Oesophagus
Plexus gastricus posterior vagi
B
A
C
C

FIG. 2.—*A.-B.* Line of incision. *C.* Flap for forming stomach pouch of Pavlov.

FIG. 1.
Gastric Vagi and Incision for Pavlov Pouch. Reproduced from Pavlov (1910).

vagus in dogs differs radically from that found in the human. Therefore it was decided as a preliminary to further work to study by actual dissection the course of the vagi in the dog in relation to pouch formation. We were unable to confirm the description given by Pavlov, observing, on the contrary, that in all essentials, the distribution was the same as in the human. Although we subsequently found that this observation had been reported several times previously in the surgical and anatomical literature (see discussion), apparently it had been overlooked or ignored by practically all physiological investigators. Since the recognition of this fact may prove vital to further quantitative investigation of the response of fundus pouches to vagal stimulation, a summary of our anatomical findings and their possible bearing on the behavior of Pavlov pouches is warranted.

Dissections were performed upon 20 dogs, all mongrels. Four of them were Pavlov pouch dogs; the remainder normal, unoperated animals. The Perman[21] method of clearance was used as an adjunct to dissection in the fresh state, but was found to offer little added clarity to nerve identification. The nerve distribution in all animals examined was the same.

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[21] Perman, E., *Acta Chir. Scand.*, 1935, **77**, Suppl. 38.

The esophageal vagi followed a simple, constant pattern. Each nerve, after supplying branches to the various thoracic viscera, divided into a ventral and a dorsal branch. Corresponding branches anastomosed, forming ventral and dorsal trunks. The union of the ventral branches occurred just distal to the base of the heart; of the dorsal, about three-quarters of an inch above the diaphragm. Small branches were supplied to the esophagus by these trunks, but plexus formation was not observed. Both trunks entered the abdomen through the esophageal hiatus in the diaphragm and passed between the 2 layers of lesser omentum.

On the stomach, the branching and course of both ventral and dorsal trunks were found to be almost exactly symmetrical (Figs. 2, 3). Slightly above the cardia, each gave one or 2 twigs to the lower esophagus, the under surface of the diaphragm, and to the cardia. Immediately below, several fair-sized branches, or one larger one which promptly divided into 2 or 3, were given off to supply the fundus and the proximal part of the body of the

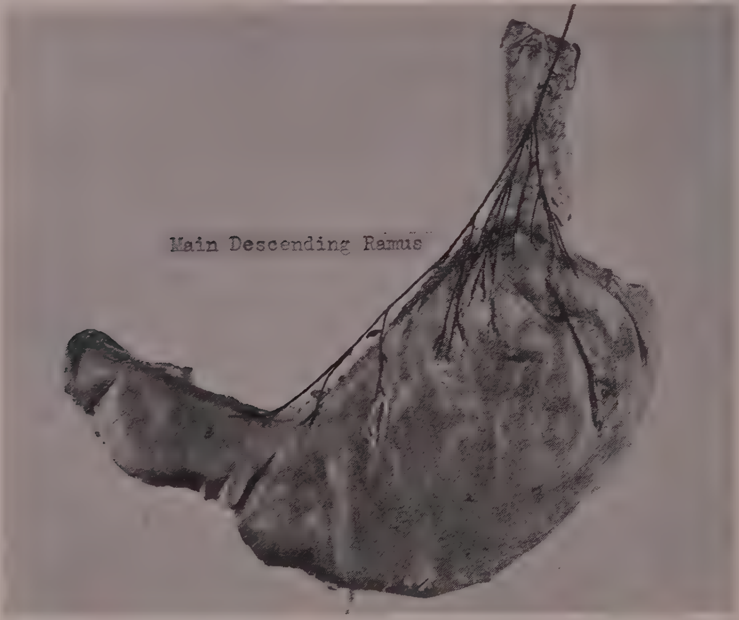

FIG. 2.

Photograph of anterior surface of dog's stomach, showing anterior vagal trunk (stained with India ink) running along lesser curvature. (Site of initiation of branching elevated, and angulation of branches increased by upward traction on nerve.)

stomach. At the same level, a large branch passed from the ventral trunk to the porta hepatis, and from the dorsal to the coeliac plexus. *Both* main trunks then descended within the omental bursa parallel to the lesser curvature of the stomach to about one inch from the pylorus. (McCrea's observation, that the gastric portion of the dorsal trunk reached the stomach, not through the omental bursa, but through the gastro-pancreatic fold, could not be verified.) Each trunk gave rise in its passage toward the pylorus to 3 to 5 more branches which passed transversely toward the greater curvature between the corresponding stomach surface and its serosal covering, to disappear in the musculature about one-third of the distance across the stomach surface. All gastric branches ran in a direction approximately at right angles to the long axis of the stomach. *No branch, great or small, was seen to traverse the stomach wall along the greater curvature.*

Neither an anterior nor a posterior gastric plexus was observed, although an occasional fine intercommunicating twig connected the fundal branches. Rarely, the main anterior and posterior descend-

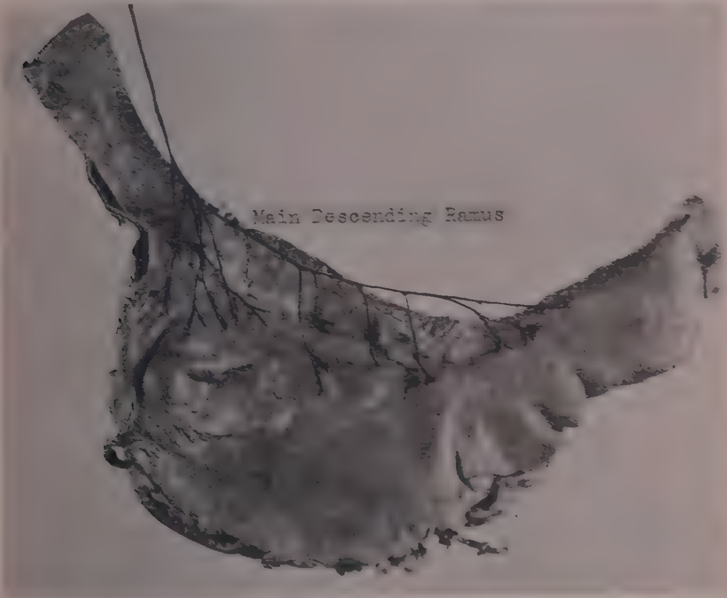

FIG. 3.

Photograph of posterior surface of dog's stomach, showing posterior vagal trunk (stained with India ink) running along lesser curvature. (Site of initiation of branching elevated, and angulation of branches increased by upward traction on nerve.)

ing rami were connected proximally by a small twig. No significant difference in size between the anterior and posterior trunks was observed, nor in the number of their branches, nor in the distance from the pylorus to which they could be followed. The pylorus was innervated by nerves of both sympathetic and vagal origin descending from the porta hepatis. At times, the hepatic branch of the anterior vagal trunk could be followed from its origin, through the hepatic portal, down to the pylorus. At other times its identity was lost in the maze of sympathetic fibers present in the region of the portal fissure.

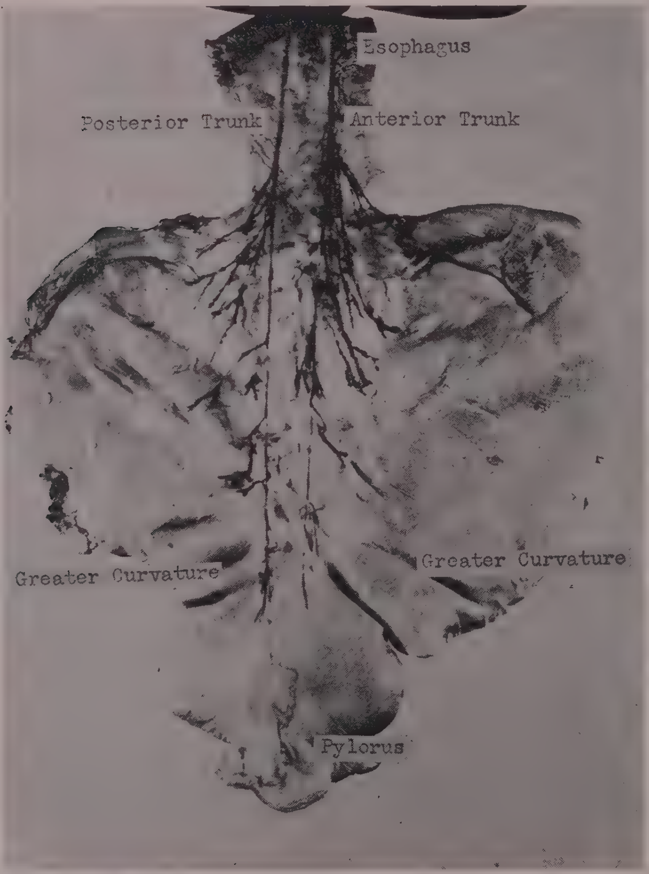

FIG. 4.

Photograph of dog's stomach, incised along greater curvature. Note symmetry of vagal trunks in their course along lesser curvature.

In addition to various minor differences, the description of Khigine and of Pavlov of the gastric distribution of the vagus nerve differs from the above in one vital respect; namely, they believed that the posterior vagal trunk coursed along the *greater curvature* of the stomach, rather than along the *lesser curvature,* symmetrically with the ventral trunk (Fig. 4). Nowhere in their work did they indicate that they had carried out actual dissection to demonstrate the course of the vagi. Instead, Khigine[22] in his original thesis, refers to Ellenberger and Baum,[23] whose description and illustration he utilized. What other literature on the gastric vagi in dogs we were able to find was entirely in accord in essential points with our observations (Latarjet,[24] Stahnke,[25] McCrea,[26] Bradley,[27] Perman[21]).

On the basis of our observations, it follows that the posterior vagal trunk does not pass uninjured through the isthmus of a pouch prepared by the Pavlov technique. On the contrary, both main trunks remain entirely outside the pouch, and roughly 75% or more of the branches from these trunks are transected by the incisions recommended by Pavlov—incisions roughly parallel to the long axis of the stomach, and hence approximately at right angles to the direction of vagal branching. Four of our dissections were done on dogs which had previously been provided with Pavlov pouches by the usual technique. In these there were found only one or 2 fine fibers crossing the stomach in the direction of the isthmus of the pouch. Not only did these branches not traverse the length of the pouch, but grossly, were not even seen to reach its isthmus. Examination of the other stomachs revealed that had Pavlov pouches been made in them, the identical condition would have resulted. Perman recognized the fact that the Pavlov technique involved the sacrifice of the bulk of the vagal supply to the pouch. Nevertheless, he accepted the pouch secretion as not being essentially different from that in other parts of the stomach, believing the sparse nerve communications on the proximal part of the pouch sufficient for this purpose. While it is possible that the few fibers which run toward the pouch isthmus might be able to supply the entire pouch through microscopic lateral branches or through the myenteric plexuses, it is no less likely that their physiological action would be limited to what anatomically seems to be their natural sphere of influence, the fundus of the pouch. In the latter case, one would be dealing with an incompletely innervated pouch, and it might be expected that the response of such a pouch to vagal stimuli would be equally incomplete.

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[22] Khigine, P. P., Inaugural Dissertation, St. Petersburg, 1894. (Communication from Dr. B. P. Babkin).

[23] Ellenberger, W., and Baum, H., *Systematische und Topographische Anatomie des Hundes,* Berlin, 1891.

[24] Latarjet, M., *Lyon Medical,* 1921, **130**, 166.

[25] Stahnke, E., *Arch. f. Klin. Chir.,* 1924, **132**, 1.

[26] McCrea, E. D., *J. Anat.,* 1924-25, **59**, 18.

[27] Bradley, O. C., *Topographical Anatomy of the Dog,* 3rd ed., New York, 1935.

Data available in the literature were insufficient to indicate whether or not Pavlov pouches did respond completely to vagal stimuli despite the anatomical considerations elucidated above. In order to determine whether there exists a physiological parallel to the observed anatomical deficiency, a technique is required for the quantitative determination of degree of vagal response. As far as we could determine, the criteria in use for pouch differentiation have only been employed qualitatively, nor do they seem to have been sufficiently refined or elaborated to permit their application to quantitative use even if so desired. The concept of "varying degree of vagal response" does not appear to have been generally appreciated, one of only 2 designations (vagal or non-vagal, Pavlov or Heidenhain) having been applied to pouches according to their response. Although in light of our anatomical findings, intermediary values of pouch response might well be expected, with the criteria as used one could not distinguish a *partial* vagal response from a *complete* one.

The authors are at present preparing pouches according to a new technique by which the vagus will be anatomically undisturbed. They propose to compare the secretory responses of this type of pouch with those of typical Pavlov and Heidenhain pouches. In this manner it is hoped that the exact status of the Pavlov pouch will be arrived at, and that standards for determining the exact functional capacity of a pouch may be formulated.

9770 P

Isolation of Poliomyelitic Virus from Human Stools.*

JAMES D. TRASK, A. J. VIGNEC AND JOHN R. PAUL. (Introduced by Francis G. Blake.)

From the Departments of Pediatrics and Medicine, Yale University School of Medicine, New Haven, Conn.

In 1912 a report from Sweden[1] described experiments in which the virus of poliomyelitis was recovered from human colonic washings obtained during the disease, and in convalescence. Sawyer[2] also reported in 1915, its recovery from the rectal washings of a convalescent case. More recently no one seems to have been able to repeat these experiments until Harmon[3] made brief mention of 5 successful attempts in which the virus was obtained from the rectal washings of 4 out of 20 patients tested; and Kramer[4] has stated that he has succeeded with similar material.

The present note also records instances in which the virus has been recovered from the stools, and is a preliminary report of the technical procedures employed in 3 of our successful experiments.

The circumstances under which the virus was isolated may be briefly described as follows: The patient was an infant (Daniel Sk., aged 18 months) who, on October 18th, 1937, had contracted a mild, febrile illness (abortive poliomyelitis) which lasted but a few days. His older brother, aged 6 years, had contracted severe, paralytic poliomyelitis 8 days previously. On the day after the onset of illness a stool was obtained from Daniel and an extract of it was subsequently inoculated intracerebrally and intraäbdominally into one monkey (8-33) which developed severe and extensive experimental poliomyelitis after a period of incubation of 7 days. Definite microscopic lesions characteristic of the experimental disease were found in the spinal cord when this monkey was sacrificed and a successful passage to monkey 8-48 was made. These results fulfill the criteria which we believe are necessary to warrant the acceptance of the result as positive. Neutralization (or reinoculation) tests, with established or fresh strains of poliomyelitic virus, have not been

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* Aided by grants from the President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Kling, C., Pettersson, A., and Wernstedt, W., *Communications Inst. méd. État Stockholm*, 1912, **3**, 5.

[2] Sawyer, W. A., *Am. J. Trop. Dis. and Prev. Med.*, 1915, **3**, 164.

[3] Harmon, P. H., *J. Am. Med. Assn.*, 1937, **109**, 1061.

[4] Kramer, S. D., personal communication.

performed because the adequacy of this procedure as a method for identifying poliomyelitic virus has been recently disputed. For the fact that different strains of poliomyelitic virus fall into different immunologic groups, renders it questionable whether these methods can at present be satisfactorily used as diagnostic tests.[5]

The technic of preparing the first stool specimen for inoculation, which was in some respects exploratory, was as follows: The stool was collected on October 19th in a pint fruit jar and was allowed to stand overnight in the refrigerator. The next morning 120 cc. of distilled water were added and the diluted stool was agitated and allowed to stand for 2 hours; 90 cc. of the supernatant fluid was then pipetted into a rubber-stoppered flask and, as a bactericidal agent, 13.5 cc. of anesthetic ether was added to make an approximate 15% suspension. This suspension was well shaken and then again placed in the refrigerator and allowed to stand overnight. On the next morning it was centrifuged at 2000 r.p.m. for one hour and the supernatant fluid below the ether-layer was then removed. (Aerobic bacterial cultures on blood-agar plates, taken from the specimen at this time, showed no growth.) To 60 cc. of this material, 1.5 cc. of normal monkey serum was added preparatory to concentration by freezing and evaporation at low temperatures in the Flosdorf-Mudd apparatus.[6, 7] The evaporating process was allowed to run for 6 hours, the original volume of 61.5 cc. being brought to 12 cc. The concentrate was then left in the refrigerator overnight, and on October 22nd half of it was inoculated into one monkey, the doses used being 1 cc. intracerebrally and 5 cc. intra-abdominally. This monkey (8-33) developed paralysis on October 29th. It had shown relatively little fever prior to the onset of paralysis. It was sacrificed on October 30th. The histological lesions were characteristic and extensive. Passage to monkey 8-48 (by cerebral and peritoneal routes) was satisfactorily accomplished.

This result has been successfully repeated on at least 2 subsequent occasions with other stool specimens obtained from this same child. The second time the virus was recovered, it was from a stool obtained on the 14th day from onset (monkey 8-49, period of incubation 6 days). This strain was successfully passed to monkey 8-96 by cerebral and peritoneal routes. The third time the virus was recovered it was from a stool obtained on the 25th day. Here a modification of the earlier technic was followed, based on the fact that in later experiments we had often found stool material, prepared in the way described, to be lethal for the monkey, particularly if inoculated intracerebrally in amounts of from 0.5 to 1.0 cc. Of 8 monkeys thus inoculated intracerebrally, 5 of them died within 3 days of the inoculation. Localized necrosis of the brain was demonstrable in 3 of these. This fact subsequently led us to limit our inoculation to the intraäbdominal route using either concentrated or unconcentrated stool extracts. In the omission of the intracerebral inoculation we were guided by the fact that we have recently found it unnecessary to use the intracerebral route invariably, in attempts to infect monkeys with human material (spinal cord) containing poliomyelitic virus.[8] So far, of 11 monkeys inoculated intraäbdominally, but one has died of peritonitis and bacteremia. The third time on which the virus of poliomyelitis was recovered from our patient Daniel Sk., the monkey (8-69) had been inoculated intraabdominally with 30 cc. of a stool extract in which the preparation of the material was approximately the same as that used with the first and second specimens, except that the extract was not concentrated. This monkey developed fever after a period of incubation of 10 days, paralyses appeared 4 days later. Extensive lesions of experimental poliomyelitis were found in the cord when the animal was sacrificed. This strain was also successfully passed by cerebral and peritoneal routes to a second monkey (8-93). Stool specimens obtained from the same child on the 43rd and 64th days from onset failed to yield the virus.

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[5] Burnet, F. M., and MacNamara, J., *Brit. J. Exp. Path.*, 1931, **12**, 57; Weyer, E. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 289; Flexner, S., *J. Am. Med. Assn.*, 1932, **99**, 1244; Paul, J. R., and Trask, J. D., *J. Exp. Med.*, 1933, **58**, 513; *Ibid.*, 1935, **61**, 447; Kramer, S. D., *Arch. Neurol. and Psychiat.*, 1935, **33**, 1371; Kessel, J. F., Van Wart, R., Fisk, R. T., and Stimpert, F. D., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 326; Howitt, B. F., *Science*, 1937, **85**, 268; Trask, J. D., Paul, J. R., Beebe, A. R., and German, W. J., *J. Exp. Med.*, 1937, **65**, 687.

[6] Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, **29**, 389.

[7] Paul, J. R., *J. Bacteriol.*, to be published.

Summary. A virus fulfiling the clinical and pathologic criteria for its identification as poliomyelitic virus has been recovered from the stools from a child with abortive poliomyelitis on the second, fourteenth and twenty-fifth days after onset. This was accomplished twice after intracerebral and intraäbdominal monkey inoculation, and once after intraäbdominal inoculation alone.

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[8] Trask, J. D., and Paul, J. R., *Science*, 1938, **87**, 44.

9771 P

Differences in Temperature of Skin and Muscles of the Lower Extremities Following Various Procedures.*

Mae Friedlander, Samuel Silbert, William Bierman and Norman Laskey. (Introduced by G. Shwartzman.)

From the Thrombo-angiitis obliterans Clinic and Department of Physical Therapy, The Mount Sinai Hospital, New York, New York.

During the past 2 years we have been investigating the regulation of the circulation in the muscles of the lower extremities. Variations in the temperature of tissues were accepted as indicating variations in the blood flow to those tissues. Thermocouples were used to determine skin surface temperatures, and the temperatures of the muscles were established by thermocouple needles inserted deep into these structures. Associated changes in body temperature were noted by means of a continuous registering rectal thermometer. In each experiment stabilization of skin, muscle, and room temperatures were obtained before any procedure was begun. Temperature observations were continued for a period of from 2 to 5 hours in each instance.

The application of heat to the upper extremities (Landis test) produced striking elevation in the surface temperature of the skin of the feet, but the muscle temperature of the legs remained unchanged. Paravertebral injections of novocaine were followed by a marked rise in skin surface temperature but no change occurred in the temperature of the muscles. Paravertebral injections of alcohol, which produce a more prolonged effect than novocaine, likewise failed to cause any elevation in calf muscle temperature although the skin surface temperature rose. Following spinal anesthesia the calf muscle temperature remained unchanged, while the temperature of the surface of the skin became markedly elevated. Intravenous injections of hypertonic sodium chloride solution were followed by elevation of both skin and muscle temperatures. Intramuscular injections of pancreatic tissue extract (Sharpe & Dohme No. 568) were not followed by any change in skin or muscle temperature. Intravenous injections of adrenalin produced a striking rise in the temperature of muscles which had a good circulation and a fall in skin temperature. Where the circulation was poor, the degree of muscle temperature elevation was less marked. It has been shown experi-

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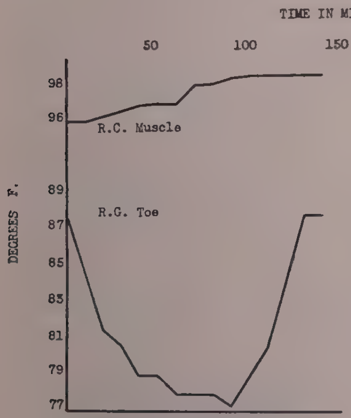
* Aided by grants from the Council of Physical Therapy of the American Medical Association.

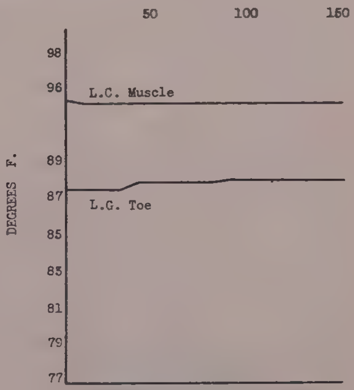
mentally by Gunning[1] and by Hoskins, Gunning and Berry[2] that adrenalin causes dilatation of the blood vessels in skeletal muscles. This work has been reviewed by Wright.[3] The data which have been collected in this investigation are to be presented in more detailed publications. One typical chart of each experiment is shown at this time.

The usual assumption that increase in skin circulation of the extremities is accompanied by an increase in muscle circulation is not substantiated by these observations. The application of heat to the forearms, and the induction of paravertebral or spinal anesthesia, while producing a marked increase in circulation of the skin, fails to influence the circulation of the muscles. Clinical observations of the failure of ganglionectomy and paravertebral injections of alcohol to relieve intermittent claudication are in accord with these findings.

Definite elevation of both skin and muscle temperatures follows intravenous injections of hypertonic salt solution. Continuous observations of the rectal temperatures during and for several hours after such intravenous injections show very slight rise (0.2° to 0.5°F.) or no rise in body temperature. The mechanism by which this increased circulation in the muscles is obtained is not yet clear. Patients with peripheral vascular disease treated with intravenous injections of hypertonic salt solution regularly show improvement



CHANGES IN SKIN AND MUSCLE TEMP.
AFTER INTRAVENOUS INJECTION OF
2CC OF 1-1000 ADRENALIN IN 500CC
OF PHYS. SALINE SOLUTION



CHANGES IN SKIN AND MUSCLE TEMP.
AFTER INTRAMUSCULAR INJECTION OF
3CC PANCREATIC TISSUE EXTRACT

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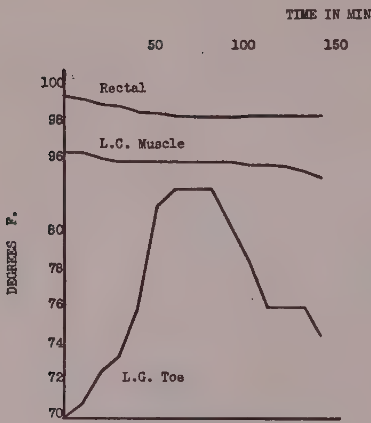
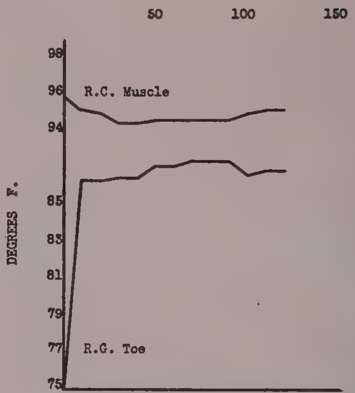
[1] Gunning, R. E. L., *Am. J. Physiol.*, 1917, **43**, 395.

[2] Hoskins, Gunning and Berry, *Am. J. Physiol.*, 1916, **41**, 513.

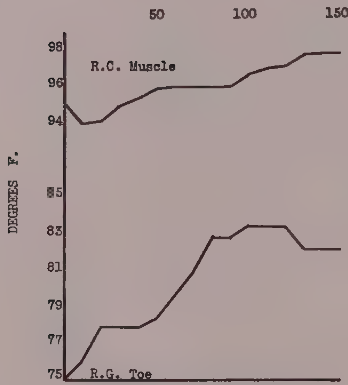
[3] Wright, *Applied Physiology*, 6th Edition, Oxford University Press, London, 1936.



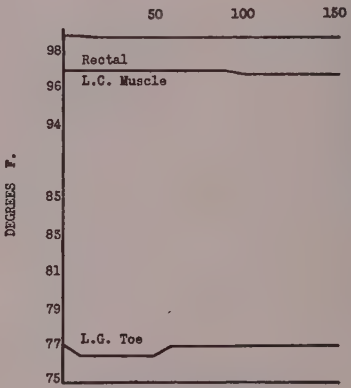
CHANGES IN SKIN AND MUSCLE TEMP.
AFTER SPINAL ANESTHESIA



CHANGES IN SKIN AND MUSCLE TEMP.
AFTER RIGHT PARAVERTEBRAL
INJECTION



CHANGES IN SKIN AND MUSCLE TEMP.
AFTER INTRAVENOUS INJECTION
300CC 5% SODIUM CHLORIDE SOL.



CHANGES IN SKIN AND MUSCLE TEMP.
AFTER INTRAVENOUS INJECTION
300CC PHYS. SALINE SOL.

in walking. The demonstration that such injections cause an elevation in the temperature of the muscles adds experimental evidence to substantiate the value of this form of treatment. From the limited number of forms of treatment that we have studied thus far, it is the only method that appears to increase the circulation in both skin and muscles.

A pronounced rise in muscle temperature is produced by intravenous injections of adrenalin. Adrenalin stimulates the sympathetic nervous system to cause vasoconstriction. This is indicated by a striking fall in skin surface temperature. The response in muscles is an elevation rather than a fall in temperature. The therapeutic possibilities of this hormone for the relief of intermittent claudication are being investigated.

It is, of course, well known that the circulation in the skeletal muscles can be increased by chemical means or in response to increased work. The purpose of this presentation is to show that such changes in circulation do not appear to be effected through a nervous mechanism.

9772

Induction of Oestrous Changes in the Monkey and Bitch by Triphenyl Ethylene.* †

J. M. ROBSON. (Introduced by A. J. Clark.)

From the Department of Pharmacology, University of Edinburgh.

It has been shown previously (Robson and Schönberg[1]) that triphenyl ethylene will induce oestrous changes in the genital organs and mating in ovariectomized mice and hypophysectomized rabbits. The effects in mice may last for several months. In the present experiment the action of this substance has been investigated in monkeys and in a bitch.

Two monkeys (*Macacus rhesus,* weighing 4.25 kg. and 3.8 kg. respectively) were ovariectomized and the first injection was given 24 and 31 days later. At the time of the first injection the sexual skin was pale in both animals. The substance was injected subcutaneously in solution in oil.

Monkey I received 1.2 gm. at the first injection, 1.0 gm. 4 days later and 1.2 gm. 8 days after the first injection. Eleven days after the first injection the whole sexual skin, including the base of the tail, became red and somewhat swollen and the redness and swelling increased during the ensuing week and was then maintained until the animal was killed for postmortem examination, 26 days after the first injection. Histological examination showed that the vaginal wall was very thick and consisted of an outer almost completely cornified zone and an inner zone of nucleated cells. The condition was similar to that produced by the injection of oestrin in the ovariecto-

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* This is part of work carried out in collaboration with Professor A. Schönberg on the activity of certain synthetic oestrogenic substances.

† The expenses of this investigation have been defrayed by a grant from the Medical Research Council.

[1] Robson, J. M., and Schönberg, A., *Nature,* 1937, **140**, 196.

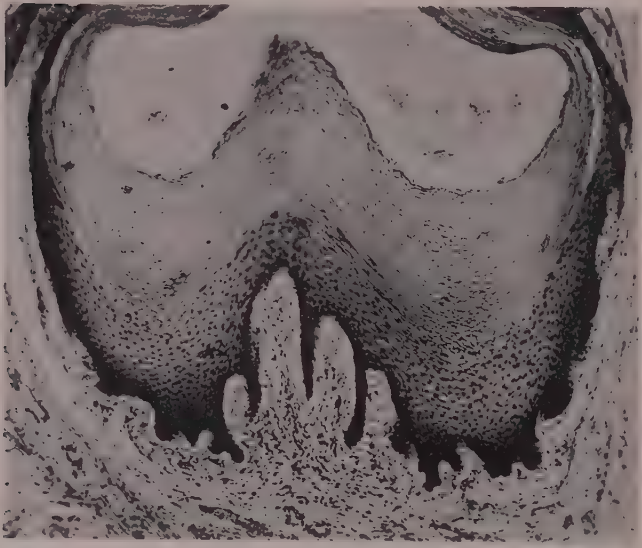

FIG. 1.
Microphotographs of sections from Monkey I treated with triphenyl ethylene.
A. Vagina. × 70.

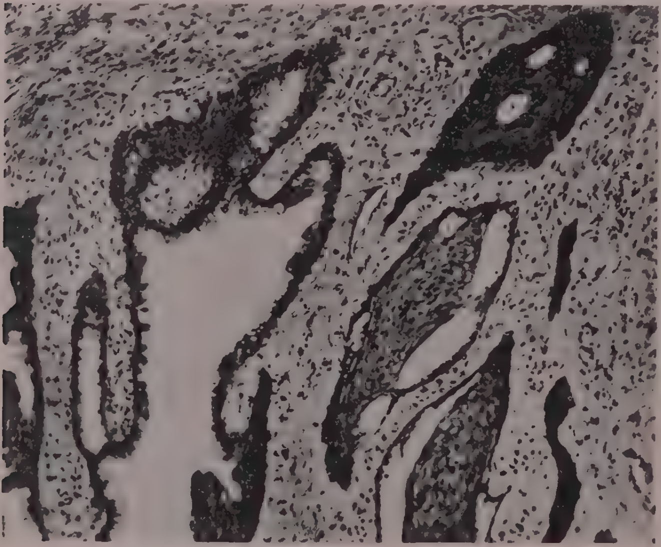

FIG. 1. B. Cervix. × 115; to show squamous metaplasia.

mized monkey (*e. g.*, Allen[2]). The cervix showed epithelial hyperplasia and also squamous metaplasia affecting especially the base of the glands such as has been described as produced by the prolonged action of oestrin (Overholser and Allen,[3] Engle and Smith,[4] Zuckerman[5]). Sections of the vagina, of the cervix and of the glandular endometrium are shown in Fig. 1.

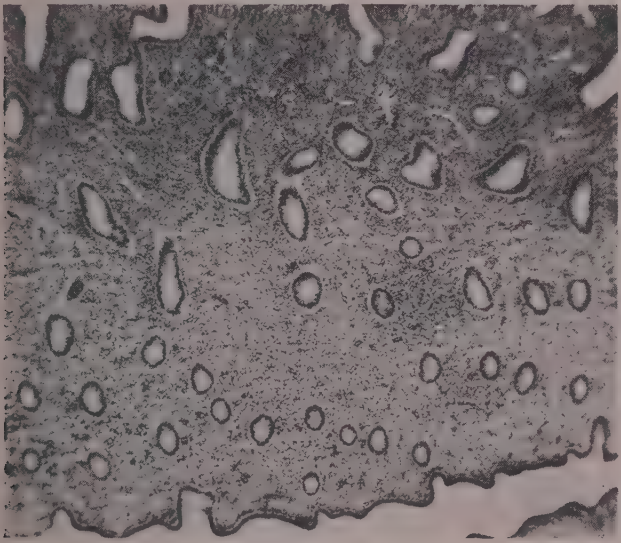

FIG. 1. C. Endometrium. × 35.

The second monkey showed typical changes in the sexual skin (redness and swelling) 7 days after the injection of 1.0 gm. of triphenyl ethylene. This animal has received further injections and is being kept for observation over a more prolonged period. It has received a total of 8.0 gm. of triphenyl ethylene over a period of 4 weeks and has shown no signs of ill health.

No sexual relations were observed when the first monkey was put with a mature male macacus after ovariectomy but before injection. After the typical sexual skin changes had been produced by triphenyl ethylene this monkey was mounted by the male. The second monkey was, however, mounted by the male both before and after the injections and hence the previous result is inconclusive.

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[2] Allen, E., *Am. J. Anat.*, 1928, **42**, 467.

[3] Overholser, M. D., and Allen, E., *Surgery, Gyn. and Obstetrics*, 1935, **60**, 129.

[4] Engle, E. T., and Smith, P. E., *Anat. Rec.*, 1935, **61**, 471.

[5] Zuckerman, S., *Lancet*, 1937, 435.

A bitch weighing 11.4 kg. and showing no signs of oestrus was ovariectomized. Five days later she received 1.0 gm. of triphenyl ethylene in oil subcutaneously. Four days after the injection some swelling of the vulva was noted and on this and on the next 2 days the vaginal smear contained a number of erythrocytes. Eleven days after the first injection 0.2 gm. of triphenyl ethylene was injected and another 0.2 gm. was given on the next day. The swelling of the vulva gradually increased and attained a maximum 14 days after the first injection; this maximum was maintained for some 10 days and then the swelling gradually decreased. Twelve days after the first injection the animal was put with a male and full mating occurred, lasting some 15 minutes. Mating occurred daily during the next 5 days, but no mating took place later on. The animal was subsequently hypophysectomized, then injected with a total of 5.8 gm. of triphenyl ethylene over a period of 16 days. No toxic effects of any kind were produced. Swelling of the vulva occurred, the animal showed sexual interest when put with the male, but no mating occurred. Eleven days after the last injection the animal was killed for postmortem examination. This showed that the removal of the pituitary had been complete. The uterus was very large, both muscle and endometrium being hypertrophied. The changes were similar to those which can be produced by the injection of oestrin in the hypophysectomized and in the ovariectomized bitch (Robson and Henderson[6]).

Experiments were made to determine whether large doses of triphenyl ethylene exerted any toxic effects. Five mature female mice received 100 mg. of the substance in 0.5 cc. oil subcutaneously. Five other mature female mice each received 10 injections (at daily intervals) of 20 mg. of triphenyl ethylene in 0.1 cc. oil. None of the animals showed any toxic effects. The second group of 5 animals was killed 9 days after the last injection and Professor Drennan very kindly examined the organs histologically (uterus, ovaries, liver, kidney, spleen, lungs, aorta, heart); no toxic changes were found. Squamous metaplasia of the endometrium was found in one of these animals (Fig. 2). This is of interest since McEuen[7] has described squamous metaplasia occurring after prolonged treatment with oestrin.

Triphenyl ethylene has a low oestrogenic activity. Its activity as estimated by vaginal cornification in mice is about 1/10,000 that of oestradiol.

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[6] Robson, J. M., and Henderson, W. R., *Proc. Roy. Soc.*, 1936, 120 B, I.

[7] McEuen, C. S., *Am. J. Cancer*, 1936, **27**, 91.

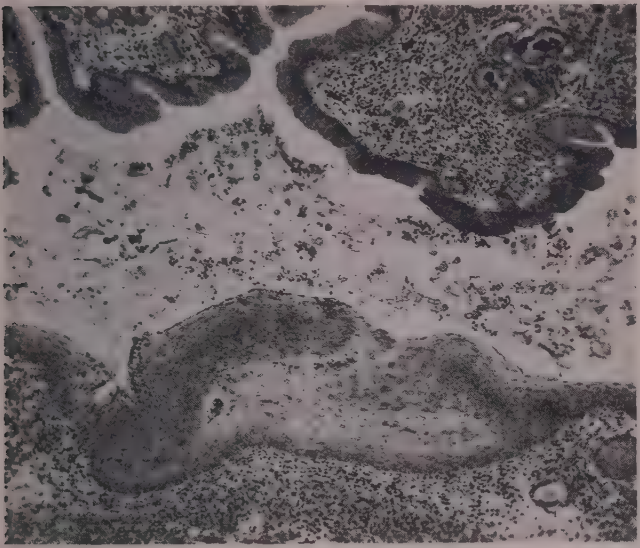

FIG. 2.
Microphotograph of section of uterus of mouse treated with triphenyl ethylene; to show squamous metaplasia. × 80.

The substance is, however, of interest for the following reasons: Its toxicity is very low since no recognizable harmful effects were produced by the following total dosages (subcutaneous injection) 5.8 gm. in the bitch; 8 gm. in a monkey; 0.2 gm. in mice; 20 gm. in a mare. In mice the tolerated dose is at least 400 times as great as the vaginal oestrogenic dose.

The ratio between the dose needed to induce mating and that required to produce vaginal cornification in the ovariectomized mouse appears to be much smaller in the case of triphenyl ethylene than in the case of oestrone.

Finally triphenyl ethylene readily produces effects of long duration and this makes it possible to maintain typical oestrous changes in castrated animals with relatively low doses.

Its activity in primates suggests a possible clinical application but this cannot be considered until experiments on carcinogenic activity, now in progress and negative up to the present, have been completed.

Summary. The administration of triphenyl ethylene to 2 ovariectomized monkeys and one ovariectomized bitch produced changes similar to those brought about by oestrin. The bitch mated. No toxic changes were obtained in these animals or in mice injected with large doses of triphenyl ethylene.

9773

Effect of Total Thyroidectomy upon Production and Maintenance of Experimental Hypertension.

FRANK GLENN AND EARL P. LASHER. (Introduced by Bruce Webster.)

From the Laboratory of Experimental Surgery, New York Hospital, and Cornell Medical College.

The effect of total thyroidectomy on the production and maintenance of experimental hypertension has been studied in 8 dogs.

In 4 animals hypertension was induced by the application of Goldblatt clamps to the renal arteries. After the stabilization of this, total thyroidectomies were done. This procedure was followed by no significant change in the level of the systolic blood pressure.

In the remaining 4 dogs total thyroidectomies were done. No change in blood pressure resulted. Constriction of the renal arteries in these animals was followed by a well sustained rise in blood pressure.

Blood cholesterol determinations were used as an index of the severity of the myxedema in both groups.

Our conclusion, therefore, is that total thyroidectomy in dogs does not effect either the production or maintenance of this type of hypertension.

9774

The Bi-Phasic Nature of Certain Alpha-Prime(?) Hemolytic Streptococci.

RALPH R. MELLON AND FRANK B. COOPER.

From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh.

It seems desirable to record these observations on variability for two reasons: first, because through them it was possible to predict the existence of a "stabilized" *viridans* phase for Group A hemolytic streptococci; and second, because sulfanilamide may be misinterpreted as curing *Streptococcus viridans* infections, which in fact are caused by hemolytic organisms temporarily stabilized in a *viridans* phase.

It is unlikely that this phenomenon would have come to our attention had it not been for the unusual type of lability possessed by the strain in question. The observations were controlled by checking culture-purity by the single-cell method on several occasions.

The strain in question (Stoddard avirulent) was grouped as an A by the Lancefield technic. Growing on neo-peptone rabbit-blood agar-plates at pH 8, it appeared to the naked eye as a hemolytic streptococcus; although at times under a 16 mm. lens a narrow rim of methemoglobin-fixation appeared about the colonies. At other times this appeared only in a few red cells, or was not detectable. The colonies were smooth and microscopically were composed of minute coccal elements, rather uniform in size and not grouped as chains.

But on proteose-peptone blood-agar plates at pH 6, the colonies were checkerman in type and surrounded by a broad zone which was grass-green in color, in contrast to the narrower dark green zone so characteristic of true *viridans* colonies. This colony showed highly pleomorphic forms, the dominant one being of a larger coccoidal type 3 to 4 microns in diameter and often forming into oidial-like chains. When the strain was transferred to a pH 8 medium the hemolytic feature became dominant, with the colonial and morphologic features described above.

For the time then, the culture could be said to exist in a green and a hemolytic phase, which could be segregated by selective environments. But these phases could not be considered as true "biotypes", otherwise they would not have been reversible; but would have tended to greater stability on repeated selection—which was not the case at all, as will be described in a more complete publication.

The fact that a green phase could be recapitulated within the variability-pattern of a Group A hemolytic streptococcus made it probable that such a phase could be stabilized temporarily in disease-conditions, and thus be mistaken perhaps for the rather distinct *Streptococcus viridans*. Although these observations were made 2 years ago, this expectation was realized on a greater scale during the current season by encountering a group of such cases clinically, whereas only an occasional one was seen before. These patients had severe sore throat; the dominant or almost exclusive organism was a streptococcus with a rather broad grass-green band, surrounding the colony. The patients responded promptly to sulfanilamide and the originally isolated colonies after a few serial transplants became characteristically hemolytic. Of especial interest was a case of hemolytic streptococcal infection of the throat whose organisms were displaced by the same type of green colony described here, within 24 hours after the administration of sulfanilamide.

Merely to be mentioned in passing, but to be described in detail later, is the fact that this rather avirulent Group A Stoddard strain has been observed to transform gradually to a Group C strain fermentatively and serologically. But before reaching this status serologically, it had only the fermentative characters of a C strain, while its antecedent was characteristically A from a fermentative standpoint. But in this intermediate state, it had a maximal virulence for mice, which was not the case for the A strain either naturally or on repeated mouse passage. Both the intermediate and the C strains were typical *beta*-hemolytic streptococci.

Summary. A certain Group A hemolytic streptococcus has been observed to recapitulate a *viridans* phase capable of selection at pH 6, but not capable of stabilization as such. Temporarily stabilized strains have, however, been observed to occur naturally in clinical cases. The prompt response to sulfanilamide might become misleading, if their true nature as hemolytic streptococci was not recognized.

9775 P

Use of Calcium Chloride in Relief of Chills Following Serum Administration.

PAUL B. BEESON AND CHARLES L. HOAGLAND. (Introduced by O. T. Avery.)

From the Hospital of The Rockefeller Institute for Medical Research.

Little attention has been devoted to the alleviation of chills, beyond such general measures as the external application of heat and the oral administration of hot fluids. In a clinical experience with chills which occasionally follow serum therapy it has become evident that these palliative measures are inadequate and that some means of prevention or control of the chill reaction would be an important adjunct to serum therapy.

In a consideration of chemical substances which might prevent or control a chill it seemed possible that soluble calcium salts, administered intravenously, might be effective. For example, calcium relieves tetany; it causes relaxation of smooth muscle, as exemplified by its clinical use in ureteral colic, bronchial asthma,[1] etc. Calcium salts are readily available and comparatively non-toxic. Their use is not considered advisable, however, in those patients having severe myocardial damage, especially those receiving digitalis, because of the known synergism between digitalis and calcium salts.[2, 3, 4]

Since in the use of calcium compounds it seems probable that the chief action is due to the existence of free calcium ion, calcium chloride was chosen for trial. With this salt the degree of ionization is high[5] as compared with the lactate or gluconate. The intravenous administration must be carried out carefully since local extravasation may cause necrosis.[6]

Administration of calcium chloride has now been carried out in 13 patients suffering chills following antipneumococcus serum therapy. As an example of the effect obtained the result in the first case may be summarized. A white woman, aged thirty, had received antipneumococcus serum intravenously in the treatment of Type II pneumonia. Thirty minutes after serum administration her skin became cold and took on a cyanotic appearance, and shortly thereafter a generalized rigor was in progress. The usual measures for symptomatic relief, consisting of blankets and the application of heat were ineffective. The intravenous administration of 10% calcium chloride solution was begun, a total of 10 cc. being injected, during a period of 4 minutes. In less than 20 seconds from the beginning of injection the chill ceased, the patient relaxed, her skin became warm, and she volunteered the statement that she was relieved. The chill did not recur.

As yet calcium chloride has not been used except in the relief of a chill already initiated. Its possible value as a prophylactic agent is also being investigated.

A summary of the cases treated with this compound is presented in Table I.

It will be noted that in all cases which had not previously received epinephrine the chill was terminated by the administration of calcium chloride, whereas in those patients who had received epinephrine little or no symptomatic relief was obtained. It is presumed that this result is related to the well-known antagonism between calcium and epinephrine.[7, 8] In these studies the maximum quantity of 10% calcium chloride to be given with safety to an adult of average weight was fixed at 20 cc. Studies are now in progress as to the mode of action of calcium in the relief of chills and concerning its action in chills associated with other processes.

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[1] Sollmann, Torald B., *A Manual of Pharmacology*, W. B. Saunders Company, 4th edition, 884-896.

[2] Bower, J. O., and Mengle, H. A. K., *J. Am. Med. Assn.*, 1936, **106**, 1151.

[3] Berliner, Kurt, *Am. J. Med. Sci.*, 1936, **191**, 117.

[4] Lieberman, Arnold L., *J. Am. Med. Assn.*, 1931, **97**, 15.

[5] Peters, John P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. 1, The Williams and Wilkins Company, 1935, 814.

[6] Seelig, M. G., *J. Am. Med. Assn.*, 1925, **84**, 1413.

TABLE I.
Summary of Clinical Experience in Relief of Chill Reactions by Intravenous Administration of Calcium Chloride.

Group	Case No.	$CaCl_2$ 10% cc.	Result
Epinephrine given during serum administration	1	10	Transient relief
	2	20	Relief with recurrence
	3	10	Transient relief
	4	20	Relief with recurrence
	5	15	No effect
No epinephrine	1	10	Complete relief
	2	15	” ”
	3	10	” ”
	4	10	Complete subjective relief, slight tremor persisted
	5	10	Complete relief
	6	10	” ”
	7	20	” ”
	8	20	” ” slight tremor persisted

Summary. The intravenous use of calcium chloride serves to alleviate chill reactions following antipneumococcus serum therapy. The use of epinephrine during serum administration appears to inhibit this favorable action of calcium.

9776 P

Segregation of “Cleavage-Substance” in the Unfertilized Egg of *Dendraster excentricus*.

A. R. MOORE.

From Hopkins Marine Station, Pacific Grove, California.

The unfertilized eggs of the sand-dollar, Dendraster, which normally are shed from the ovaries fully mature, were sedimented by

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[7] Salant, William, and Vogt, Elkin, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 681.

[8] Jendrassik, L., and Czike, A., *Biochem. Z.*, 1928, **193**, 285.

centrifuging at approximately 40,000 gravities for 7-10 minutes, with the result that the eggs were elongated to dumb-bell-shape and pear-shape and the nucleus thrown to the extreme centripetal end, against the "oil-cap". The eggs were then caused to develop parthenogenetically by means of a modified Loeb's method of acidulated sea water and hypertonic treatment. With respect to the effects of sedimentation on subsequent development, the eggs of different individuals behaved somewhat differently. On the basis of types of cleavage the females whose eggs were used may be classed in three groups, namely, (1) those whose eggs show complete but unequal segmentation, cleavage of the centripetal (nucleated) end being retarded so that as a result the blastomeres are abnormally large there, rapid cleavage at the centrifugal end resulting in blastomeres of approximately normal size; (2) those in whose eggs the centripetal half (with egg-nucleus) shows nuclear division and the formation of multiple asters, without cytoplasmic division, but the non-nucleated cytoplasm of the centrifugal half cleaves regularly, forming normal-appearing blastomeres; (3) those in whose eggs the egg-nucleus at the centripetal end moves inward and enlarges but does not divide, only the centrifugal (non-nucleated) half of the egg showing cytasters and regular cytoplasmic division. Assuming that cleavage of the cytoplasm depends upon the presence of a substance carried by heavy structures in the cytoplasm, then the 3 typical results may be understood if we suppose that in (1) the heavy structures bearing cleavage-substance are only slightly segregated by the centrifugal force applied, since the centripetal end, while it does segment, is greatly retarded, that in (2) there has occurred segregation of cleavage-substance sufficient to inhibit cytoplasmic division in the centripetal half but nuclear division still occurs, and that in (3) there is complete segregation of cleavage-substance in the centrifugal half, since even nuclear division fails in the centripetal half. The appearance of cytasters and nuclear division in the centripetal half in (2) without cleavage of the oöplasm of that half would suggest the elimination of centrosomes or centrioles as bearers of the substance responsible for cleavage of the oöplasm. But such cleavage-substance has been completely segregated in the non-nucleated centrifugal half in groups (2) and (3).

9777

Vitamin C Content of Human Non-Scorbutic Cerebrospinal Fluids.

W. W. JETTER AND T. S. BUMBALO. (Introduced by Guy E. Youngburg.)

From the Department of Biological Chemistry, University of Buffalo Medical School, and the Department of Pediatrics, Buffalo City Hospital.

Much data have been accumulated regarding the ascorbic acid content in various body fluids and tissues. Little information is to be found concerning the occurrence of vitamin C in the cerebrospinal fluid, and none in the English or American literature. Kasahara and his coworkers[1, 2] and Plaut and Bulow[3, 4] have shown that vitamin C is present in the cerebrospinal fluid of experimental animals. The latter[5] and Monauni[6] have determined the ascorbic acid content of the cerebrospinal fluid of psychiatric patients. Monauni observed seasonal variations and considered 0.5 to 0.8 mg. % as normal for winter and summer months respectively. In an effort to obtain further data, the authors have determined the vitamin C reducing power of the cerebrospinal fluid in 50 ambulatory patients who gave no clinical evidence of avitaminosis C.

The chemical procedure used was the dichlorophenol indophenol method, the technique being a modification of that used by the authors for work with urine.[7, 8] Because of the low protein content of the cerebrospinal fluid, direct titration is possible without preliminary removal of the protein. Two cc. of the fluid are brought to a total volume of 5 cc. with water and adjusted to an approximate pH of 4.7 with 5% acetic acid. If smaller quantities of the fluid are used, the final volume is reduced proportionately. The solution is then titrated with 2:6 dichlorophenolindophenol (2 mg. in 50 cc. of water, one cc. of which is equivalent to 0.02 mg. of ascorbic acid) to a faint bluish red endpoint. 92-100% of ascorbic acid when added in quantities equal to its usual concentration in cerebrospinal fluid was recovered from the fluid by this method.

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[1] Kasahara, M., and Gammo, H., *Z. f. d. ges. Neurol. u. Psychiat.*, 1937, **157**, 147.

[2] Kasahara, M., Tatsumi, M., and Gammo, H., *Ibid.*, 1937, **157**, 149.

[3] Plaut, F., and Bulow, M., *Klin. Wchnschr.*, 1935, **14**, 1716.

[4] Plaut, F., and Bulow, M., *Ibid.*, 1935, **14**, 1318.

[5] Plaut, F., and Bulow, M., *Z. f. d. ges. Neurol. u. Psychiat.*, 1936, **154**, 481.

[6] Monauni, J., *Ibid.*, 1937, **157**, 636.

[7] Jetter, W. W., and Bumbalo, T. S., *Am. J. Med. Sc.*, in press.

[8] Bumbalo, T. S., *Am. J. Dis. Child.*, in press.

TABLE I.
Ascorbic Acid Content of Cerebrospinal Fluid in 50 Cases Showing No Avitaminosis C.

Range $\frac{\text{mg.}}{\text{100 cc.}}$	0.7-1.0	1.1-1.3	1.4-1.6	1.9	2.1
No. of observations	12	20	14	3	1

Thirty-three of the 50 cases were syphilitics. All were under active treatment in the outpatient department and were admitted for routine spinal examination. Involvement of the central nervous system, when present, was asymptomatic. The remaining 17 cases exhibited various types of pathology but all were ambulatory. In these cases, examination of the cerebrospinal fluid was not indicated, the procedure being performed solely for the determination of the ascorbic acid content. As far as could be ascertained, the diets of the patients were adequate in vitamin C. In all of the cases, samples of the cerebrospinal fluid were obtained not later than 3 days after entry to the hospital. The hospital diet contained an adequate ration of vitamin C. The diet before hospitalization was representative of that usually followed by individuals of the class attending the outpatient department of a large city hospital.

Clinical examination revealed no evidence of acute infection in any member of the group. Also, no clinical manifestations of avitaminosis C could be detected. In view of the minimal pathology in all cases, the absence of acute infection and any clinical manifestation of avitaminosis C, we believe that the group may be considered as representative of normal for the purpose of this paper.

The state of nutrition was determined by the clinical condition of the patient and was classified as good, fair or poor. No cases showing definite malnutrition were included in the series. The nutrition was considered good in 26 cases; fair in 18, and poor in 6. No definite correlation between the state of nutrition and the ascorbic acid content of the cerebrospinal fluid could be detected. The majority of cases fell in an age group from 25-65 years. No correlation between age and vitamin C was found.

The ascorbic acid content of the cerebrospinal varied in this series from 0.7 to 2.1 mg. %. Thirty-one fell in the group between 0.9 and 1.4 mg. %. The median of the entire series was 1.2 mg. %. This level of cerebrospinal ascorbic acid of so-called normal individuals is somewhat higher than that reported by Monauni.[6] As yet we have no data establishing a definite level below which symptoms of avitaminosis C will occur. Further work in conjunction with studies of the urinary excretion is indicated. The determination of the vitamin C content of the cerebrospinal fluid may become an aid in the diagnosis of avitaminotic states.

Conclusion. The ascorbic acid content of the cerebrospinal fluid in a series of 50 ambulatory patients has been determined. All individuals were on a diet adequate in vitamin C and showed no clinical evidence of avitaminosis C or of acute infection. The ascorbic acid content varied from 0.7 to 2.1 mg. % with a median of 1.2 mg. %.

9778 P

A New Goiter-Producing Diet for the Rat.

GEORGE R. SHARPLESS. (Introduced by F. W. Hartman.)
From the Henry Ford Hospital, Detroit.

Diets which consistently produce goiter in rats have been limited to those composed of natural food materials. Modifications of the Steenbock rachitogenic diet have been used successfully by Levine, Remington and von Kolnitz,[1] Thompson,[2] and others. Hellwig[3] has used a diet of 50% rolled oats and 50% corn meal boiled in distilled water. Other diets, composed mostly of the cereal grains or their milling products, have been used with little success.

Attempts to produce goiter in rats with the above diets or modifications of them resulted in failure. It was found that rats of the same colony and under the same environmental conditions that resulted in failure with other diets, readily developed goiter when fed the following diet: soy bean flour (unprocessed) 75.0 parts, dried yeast 3.0 parts, butter fat 5.0 parts, sodium chloride 1.0 part, calcium carbonate 0.5 part, sucrose 15.5 parts, and viosterol 15 drops per kilo of food.

Rats weighing 50-70 gm. were used in the study. More than 150 were fed the diet over a period of 2 years. Enlarged glands were obtained consistently. After 4 weeks on the diet, the glands of many animals were enlarged to 5 times normal size but there were wide individual variations. The weight of the gland in relation to body weight after 8 weeks was rarely greater than the largest glands of the younger group, but the individual variation was much less. The

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[1] Levine, Harold, Remington, R. E., and von Kolnitz, Harry, *J. Nutrition*, 1933, **6**, 325.

[2] Thompson, Juanita, *J. Nutrition*, 1932, **5**, 359.

[3] Hellwig, C. A., *Endocrinol.*, 1934, **18**, 197.

TABLE I.

Weight and Iodine Content of Thyroids of Normal Rats, and Those Fed the Goiterogenic Diet with and without Iodine.

Diet	Iodine content of diet, gamma per kilo	Age when killed, days	No. of animals	Aver. Thyroid Wt.			Dry matter in thyroid %	Iodine conc. dry basis %	Total iodine in each gland gamma
				Fresh		Dry			
				Total mg.	Per 100 gm. body wt. mg.	Total mg.			
Stock	120	75	14	19.1	11.2	4.9	25.5	.0625	3.06
Goiterogenic (No. 153)	60-80	57	10	32.4	29.3	6.9	21.2	.0092	0.64
,, (No. 153)	60-80	85	17	65.5	47.0	13.4	20.5	.0055	0.74
No. 153 with KI	430-450	85	14	16.8	11.2	5.0	29.6	.233	11.66

average enlargement obtained after 4 weeks and after 8 weeks in 2 representative groups where iodine determinations were made is shown in Table I.

That iodine added to the diet as potassium iodide completely prevented the enlargement is also shown in Table I. The glands were more firm and contained less moisture than those of normal control animals. Iodine was determined by the method of Trevorrow and Fashena.[4] Four or more goiterous glands were combined for a single determination. Other glands were analyzed individually. The iodine content of the goiterous glands is practically the same as that found by Levine, Remington and von Kolnitz[1] in glands of similar size.

Microscopically the enlarged glands are hyperplastic with little or no colloid. The lumen of the acini is small and almost indistinguishable in large areas. The lining cells are large. The glands of stock animals and of those fed the goiterogenic diet with potassium iodide are normal. The latter contain more colloid.

By adding soy beans to the diet of young rats McCarrison[5] produced an enlargement of the thyroid which was not prevented by potassium iodide. Under the conditions of the present study, McCarrison's observation was not confirmed. However, the iodine content of the goiterogenic diet was 4 times the 14 gamma per kilo found by Levine, Remington and von Kolnitz[1] in diets causing a similar degree of enlargement. Whether the diet contains a factor which affects the utilization of iodine may be indicated by experiments in progress. A similar degree of enlargement was not obtained when heated soy bean flour was substituted for the unprocessed flour.

Summary. In 4 to 8 weeks, a diet containing 75% raw soy flour induces in the thyroid of young rats an enlargement of 3 to 5 times normal. The enlargement is accompanied by hyperplasia and loss of colloid. Although the diet contains more iodine than would be expected, additional iodine will completely prevent the enlargement.

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[4] Trevorrow, V., and Fashena, A. J., *J. Biol. Chem.*, 1935, **110**, 29.

[5] McCarrison, R., *Ind. J. Med. Res.*, 1933, **21**, 179.

9779 P

Effect of Respiratory Stimulating Factors on Endogenous Respiration of Yeast.

E. S. Cook, Sister Mary Jane Hart, O. P., and Russell A. Joly. (Introduced by S. Tashiro.)

From the Institutum Divi Thomae, Graduate School of Scientific Research, Cincinnati, Ohio.

Previous papers from this laboratory have reported the preparation from yeast[1, 2, 3] and malt-combings,[4] of factors which stimulate the respiration of yeast. In the above studies the stimulating substances were tested by the Warburg manometric technique using Ringer-phosphate-glucose as the suspending medium for the yeast. Under these conditions the respiratory stimulating factor (R.S.F.) could act by increasing the utilization of exogenous glucose. Hence, it seemed desirable to determine the activity of the R.S.F. on yeast suspended in sugar-free media. It has been shown by Stier and Stannard[5] that in the absence of exogenous glucose the oxygen uptake of a yeast suspension represents true respiration uncomplicated by fermentation and is properly called endogenous respiration rather than autofermentation. Under these conditions the carbohydrate reserves (glycogen) of the cell are utilized.

In the present experiments Fleischmann's bakers' yeast *(Saccharomyces cerevisiæ)* from the center of a cake was suspended in Ringer-phosphate and Ringer-phosphate-glucose (0.02% glucose) at a pH of 7.3. Potassium phosphate at the same pH was also used as a suspending medium with similar results, but lowering the pH to 4.3 somewhat decreased the respiration. A preliminary centrifugal washing with the suspending medium was carried out in some experiments but seemed to be unnecessary. A final yeast count of 250 (count x 250,000 = cells/cc.), determined with Beck's photodensitometer,[6] was used in all experiments. At a temperature of 37.5°C. and after a 15-minute equilibrium period in air, the oxygen

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[1] Norris, R. J., and Ruddy, Sr. M. Veronita, *Studies Inst. Divi Thomae*, 1937, **1**, 53.

[2] Norris, R. J., and Hart, Sr. M. Jane, *Ibid.*, 1937, **1**, 65.

[3] Cook, E. S., and Kreke, C. W., to be published.

[4] Norris, R. J., and Kreke, C. W., *Studies Inst. Divi Thomae*, 1937, **1**, 137.

[5] Stier, T. J. B., and Stannard, J. N., *J. Gen. Physiol.*, 1936, **19**, 461, 479; *J. Cell. and Comp. Physiol.*, 1937, **10**, 79.

[6] Beck, W. A., *Science*, 1937, **85**, 2206.

uptake was measured for a 25-minute period. As is well known,[7] the oxygen uptake in the presence of glucose is much higher than in glucose-free media, and we found that an increase in the glucose concentration above 0.02% caused too great an oxygen consumption to be read under the conditions of the experiments.

R.S.F. preparations from yeast[3] and malt-combings[4] were added in concentrations of 0.5 mg. to 1.0 mg. per cc. The results of 36 determinations indicated that the respiration was increased both in the presence and absence of glucose and to the same extent in both cases. In these experiments the maximum variation was 18% and the average variation 6%, which is within experimental error. Data from a typical experiment are given in Table I. Stimulation of 150% to 250% has been obtained with yeast of varying age and with varying R.S.F. preparations. Similar results were obtained when a pure 48-hour culture of yeast was used but the degree of stimulation was lower. This was not wholly unexpected and is receiving further investigation.

TABLE I.
Respiration Stimulation by 1 mg./cc. of R.S.F. Sample 15.

	Ringer	Ringer+R.S.F.	Ringer+Glucose	Ringer+Glucose+R.S.F.
Cmm. O_2 absorbed	79.1	149.26	111.65	207.41
% stimulation		85.03		85.7

The R.S.F. preparations were also examined in Ringer-phosphate solutions with and without glucose but in the absence of yeast. No oxygen uptake was observed even when the respiration period was extended to 2 hours. Furthermore, no oxygen consumption occurred when R.S.F. preparations were added to suspensions of yeast killed by autoclaving. Thus, it is seen that the R.S.F. preparations do not, of themselves, "respire", nor are they capable of oxidizing glucose in the absence of the living cell.

Summary. Respiratory stimulating factors, obtained from yeast and malt-combings, stimulate the respiration of yeast to the same degree in the absence of exogenous glucose as in its presence. These factors under the same conditions but in the absence of living yeast, cause no oxygen uptake. Hence, the factors stimulate the true respiration of yeast. These studies are being extended.

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[7] *Cf.* Warburg, O., *Biochem. Z.*, 1927, **189**, 354; Meyerhof, O., and Iwasaki, K., *Ibid.*, 1930, **226**, 16.

9780

A Note on the Color Test for Pentoses.

HENRY TAUBER.

From the Research Laboratories of the McLeod Infirmary, Florence, S. C.

With the benzidine color test recently described[1] the following glucuronates* have been tested; borneol glucuronic acid, menthol glucuronic acid, and glucuronic acid monobenzoate. When 20 mg. samples were employed the test was negative, whereas Bial's test was positive with 2 mg. except in the case of borneol glucuronic acid which showed a positive test with Bial's reagent only when large amounts were employed. Glucuronic acid and glucuronic acid lactone gave a positive test with more than 0.2 mg. In this respect the benzidine test resembles Bial's test.

The new test is based on the formation of furfural. This aldehyd gives a red color with the benzidine solution without boiling.

In view of the fact that glucuronic acid does not occur free in urine, but in combination with phenols, etc., the benzidine test may be used to differentiate between pentoses and glucuronates.

9781

Effect of pH upon Metamorphosing Action of Thyroxine on Tadpoles.

S. H. ROSEN. (Introduced by D. Marine.)

From the Laboratory Division, Montefiore Hospital, New York City.

The basis for the present study was an observation made in experiments performed in the spring of 1935 to investigate the finding of Zondek and Reiter[1] that $CaCl_2$ inhibited and KCl accelerated the metamorphosing action of thyroxine on tadpoles. It was observed that, while $CaCl_2$, Ca gluconate and KCl media, all with the same pH, 6.4-6.8, as our tap water control medium, had no effect upon the metamorphosing action of thyroxine.* alkaline Na phosphate

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[1] Tauber, H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 600.

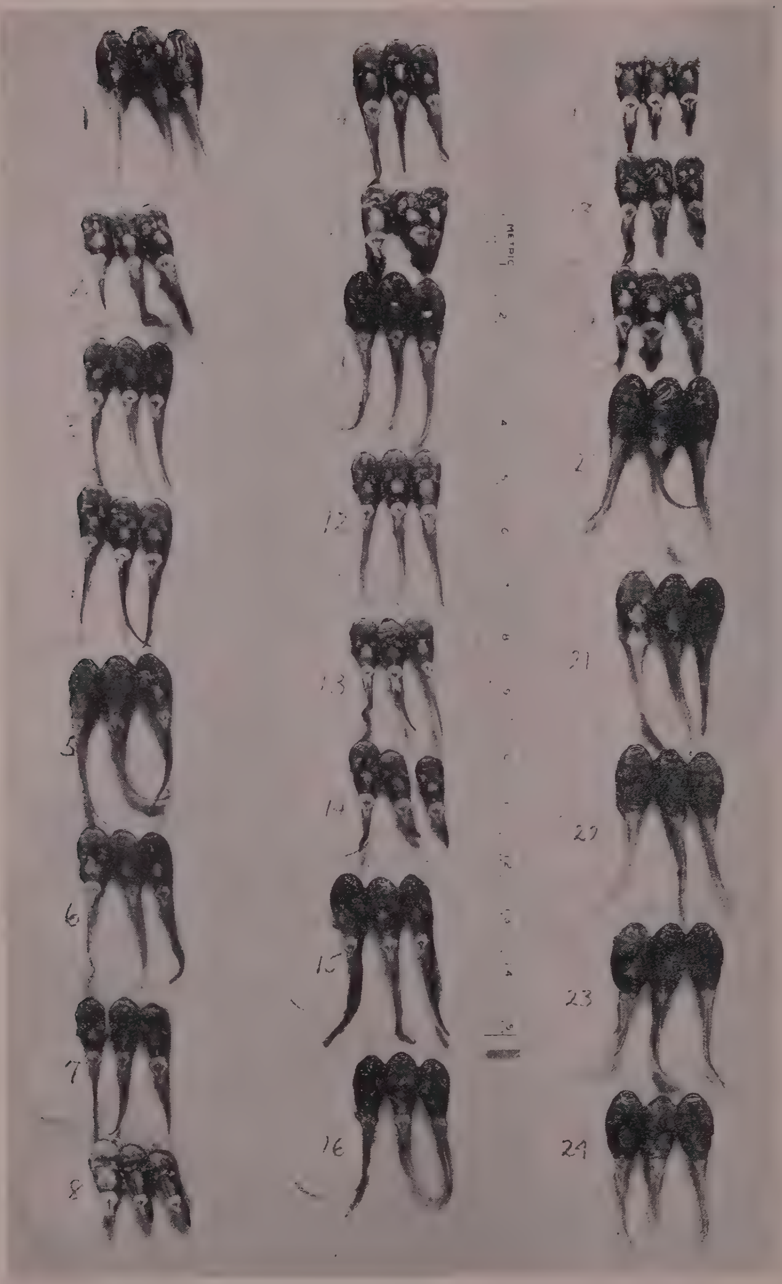
* I am indebted to Dr. A. J. Quick for samples of glucuronates.

[1] Zondek, H., and Reiter, T., *Klin. Wschr.*, 1923, **2**, 1344.

* Kosmin and Resnicenko[3] had also obtained negative results with $CaCl_2$ and KCl, and Hellwig[4] subsequently obtained negative results with $CaCl_2$.

[3] Kosmin, N. P., and Resnicenko, M. S., *Trudy laboratorii eksperimentalnoj biologii Moskovskogo zooparka*, 1927, **3**, 9.

[4] Hellwig, C. A., *J. Lab. and Clin. Med.*, 1936, **21**, 1131.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Figs. 1-24.

Group	Thyroxine	Solutions Tested	%	pH
1	—	—		6.4
2	1:20 million	—		6.4
3	”	$Na_2HPO_4 \cdot 12H_2O$	.036	8.2
4	”	”	.36	8.8
5	”	$Na_3PO_4 \cdot 12H_2O$	.12	11.0
		$NaH_2PO_4 \cdot H_2O$	.035	
6	”	$Na_2HPO_4 \cdot 12H_2O$	.2	8.4-8.6
7	”	”	.08	6.6-6.8
		$NaH_2PO_4 \cdot H_2O$	.046	
8	”	”	.078	5.0-5.2
9	”	$Na_2HPO_4 \cdot 12H_2O$	.2	6.8
		HCl		
10	”	”	.2	4.8-5.0
11	”	$CaCO_3$ saturated sol.		10.0
12	”	” ” ”		8.0
		HCl		
13	”	$CaCO_2$ saturated sol.		6.4-6.6
		HCl		
14	”	$CaCO_2$ saturated sol.		4.8-5.0
		HCl		
15	”	Na_2CO_3	.012	10.0
		$NaHCO_3$	.062	
16	”	$3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$ saturated sol.		10.0
17	”	Citric acid		4.8-5.0
18	”	Acetic acid		5.0
19	”	HCl		4.8-5.0
20	—	$Na_3PO_4 \cdot 12H_2O$	.12	11.0
		$NaH_2PO_4 \cdot H_2O$	.035	
21	—	$CaCO_3$ saturated sol.		10.0
22	—	$NaH_2PO_4 \cdot H_2O$	0.1	5.0
23	—	Acetic acid		5.0
24	—	HCl		5.0

media, pH 8.4 and 9.6, inhibited, and acid Na phosphate media, pH 5.4, accelerated the action of thyroxine. Abelin[2] had obtained a similar inhibiting effect, although not consistently, with the alkaline phosphate Na_2HPO_4, but the acid phosphate, NaH_2PO_4, had no effect; and he implied that a control experiment with another alkaline salt, $NaHCO_3$, gave negative results. He, therefore, attributed the inhibiting effect of Na_2HPO_4 to the PO_4 ion. Our findings, however, indicated that the effects of the phosphate media were dependent upon their pH, and suggested that alkalinity and acidity, *per se,* caused inhibition and acceleration of the metamorphosing action of thyroxine irrespective of particular ions. To demonstrate the latter, experiments were performed, in the spring of 1937, in which a number of different alkaline and acid solutions were employed.

The tadpoles *(Rana sylvatica)* were taken from a single brook. For the experiments groups of 6 or 7 of uniform size and stage of development (no extremities and large tails) were transferred to small enamel dishes. The following was the daily procedure. From about 9 A.M. to 1 P.M. the tadpoles were fed grated egg white in fresh tap water. At about 1 P.M. the tap water was again changed, and the thyroxine and solutions to be tested added so that the final volume in each dish was 400 cc. The pH was then determined colorimetrically. The dishes were kept at room temperature exposed to daylight during the experimental period of 5 days. The usual criteria of thyroid action on tadpoles, namely, hastened differentiation (growth of extremities, tail absorption, etc.) and emaciation, were employed. Thyroxine, freshly dissolved in distilled water with the aid of 1 or 2 drops of 10% NaOH at the beginning of the experiments, was used in the tadpole media in final concentration of 1 to 20 million. The possibility that thyroxine might precipitate out of the acid media during the 20-hour daily test period was ruled out by tests in which even more acid solutions, pH 3 to 4, and greater concentrations of thyroxine, 1 mg. in 1 liter or 1 to 1 million, were employed.

Our earlier observations on the effects of alkaline and acid phosphates were confirmed and extended. The alkaline phosphate, Na_2HPO_4, pH 8.2-8.8, retarded the metamorphosing action of thyroxine (Figs. 3, 4, 6). There was no appreciable difference in the degree of retardation dependent upon the concentration of the salt; this is at variance with the finding of Abelin[2] that lower concentrations of Na_2HPO_4 were more effective. A mixture of Na_3PO_4 and NaH_2PO_4, pH 11, (Fig. 5) caused slightly greater retardation of the thyroxine action than did Na_2HPO_4, pH 8.2-8.8. The acid phosphate, NaH_2PO_4, pH 5.0-5.2, on the other hand, definitely accelerated the metamorphosing action of thyroxine (Fig. 8).

Confirmatory evidence that the different effects of the above phosphates were dependent solely upon the pH of the solutions was obtained from the following 2 experiments. The phosphates Na_3PO_4, Na_2HPO_4 and NaH_2PO_4 were employed in such combinations and concentrations as to give the following range of pH: 11, 8.4-8.6, 6.6-6.8, and 5.0-5.2, while containing in every instance the same amount of P, namely 70 mg. (Figs. 5, 6, 7, 8). Comparison of the photographs of these groups with that of the tap water thyroxine control (Fig. 2) reveals a difference, as well as a gradation of effects ranging from retardation of the thyroxine action on the alkaline side to acceleration on the acid side; while in the phosphate combination with the pH 6.6-6.8 the tadpoles show almost the same degree of differentiation and emaciation as in the tap water thyroxine control pH 6.4.

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[2] Abelin, I., *Klin. Wschr.*, 1923, **2**, 1650.

The second experiment was the following. Na_2HPO_4 was used in the same final concentration, namely, 0.2% or 70 mg. P, in 3 dishes. One, to which nothing further was added, had a pH of 8.4-8.6. To the second and third, HCl was added to bring the pH to 6.8 and 4.8-5.0 respectively (Figs. 6, 9, 10). The range of effects from retardation of the thyroxine action in the alkaline dish to acceleration in the acid dish, and absence of, or only slight retarding effect in the second dish with a pH only slightly above the tap water-thyroxine control, pH 6.4, is clearly demonstrated.

The effect of hydrogen ion concentration was similarly demonstrated with another salt (Figs. 11, 12, 13, 14). By the addition of increasing amounts of HCl to a fixed amount of saturated $CaCO_3$, a range of pH of 10, 8.0, 6.4-6.6, and 4.8-5.0 was obtained, while maintaining in each instance the same amount of Ca, namely 2.1 mg. As with the phosphate so treated, a range of effects was obtained from retardation of the thyroxine action on the alkaline side to acceleration on the acid side; while at pH 6.4-6.6 there was no effect upon the thyroxine action when compared with the tap water thyroxine control of the same pH.

Further evidence that alkalinity retards and acidity accelerates the metamorphosing action of thyroxine, irrespective of the ions employed, was obtained by the use of a number of different salts and acids, namely, Na_2CO_3 and $NaHCO_3$, pH 10 (Fig. 15), saturated $MgCO_3$, pH 10 (Fig. 16), citric acid, pH 4.8-5.0 (Fig. 17) acetic acid, pH 5.0 (Fig. 18), HCl, pH 4.8-5.0 (Fig. 19). In every instance the alkaline salts definitely retarded, and the acids definitely accelerated the action of thyroxine.† Also significant was the observation that groups of tadpoles subjected to the influence of thyroxine at the same, or nearly the same pH, regardless of the composition of the medium, showed similarity in size and stage of metamorphosis.

That acidity and alkalinity, *per se*, had no effect, in the 5-day experimental period, upon the growth or metamorphosis of the tadpoles was demonstrated by the use of some of the alkaline and acid media without thyroxine, namely, Na_3PO_4 and NaH_2PO_4, pH 11

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† The observation of Brandt[5] that the water of the Eugenquelle in Bad Kudowa, a German spa, inhibited the metamorphosing action of thyroxine and desiccated thyroid on axolotls, apparently supports this finding with respect to the effect of alkalinity. Although Brandt attributed the inhibiting effect to "the biological action of the combination of all the constituents" of the Eugenquelle water, the fact that this water is rich in alkaline salts, and that its free CO_2 was allowed to escape before it was used, makes it probable that the inhibiting effect was due to alkalinity of the water.

[5] Brandt, W., *Z. f. d. ges. exp. Med.*, 1936, **99**, 478.

(Fig. 20), saturated $CaCO_3$, pH 10 (Fig. 21), NaH_2PO_4, pH 5.0 (Fig. 22), acetic acid, pH 5 (Fig. 23), and HCl, pH 5 (Fig. 24).

Conclusion. Acidity accelerates and alkalinity retards the metamorphosing action of thyroxine on tadpoles.

9782 P

Action of Ultra-violet on Members of the *Pseudomonas fluorescens* Group.

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The early theory of the destructive action of ultra-violet rays on bacteria was that hydrogen peroxide was produced in the medium. Oker-Blom[1] proved that the death of the bacteria was not caused by hydrogen peroxide, but by the direct action of the rays on the protoplasm of the organism. Burge and Neill[2] found fluorescent organisms to be more resistant to ultra-violet than colon organisms. They attributed the superior resistance of the members of the fluorescent group to their power of converting the short waves into long ones, and thereby escaping the coagulative action of the short waves on the protoplasm.

The investigation reported in this paper was undertaken to determine whether the pigment produced by organisms of the genus *Pseudomonas* protects these organisms from radiant energy furnished by a quartz mercury-vapor lamp.

A suitable medium for irradiation was first considered. Both plain broth and lactose broth were found unsuitable because they protected the organism from the effects of the ultra-violet. The synthetic asparagine medium proposed by Georgia and Poe[3] was found to be satisfactory.

In the asparagine medium, 12-, 24-, 36-, and 48-hour cultures of several members of the *Pseudomonas* group were prepared; 10 cc. of each culture were diluted to 100 cc. with sterile water, and 4 cc. of the diluted culture were exposed to the vertical rays of a Gallois mercury-vapor lamp from one to 40 minutes. Undiluted cultures were also tested. Appropriate dilutions of the irradiated cultures were made and plated in nutrient agar. The colonies were counted and calculations were made showing the number of survivors per hundred million of the original cultures. Cultures of *A. aërogenes* and *E. coli* were treated in a similar manner. Before these cultures were irradiated, they were diluted with asparagine medium until the bacterial content was approximately the same as that of the *Pseudomonas* cultures. The bacterial population in all cultures was about 50,000,000 per cc. Some of the average results are reported in Table I.

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[1] Oker-Blom, Max, *Z. f. Hyg.*, 1913, **74**, 242.

[2] Burge, W. E., and Neill, A. J., *Am. J. Physiol.*, 1915, **38**, 399.

[3] Georgia, F. R., and Poe, C. F., *J. Bact.*, 1931, **22**, 249.

TABLE I.
Survival Data when Cultures Were Exposed to Ultra-violet. (Survivors per 100,000,000.)

Minutes	*A. aërogenes*	*E. coli*	Pseud. organisms Aver. of 4
		12-hr. culture	
1	990,000	435,000	8,210,000*
3	285,000	6,900	1,400,000
5	47,000	3,700	111,700
7	5,550	1,600	17,400
10	3,950	80	2,100
20	420	30	410
40	145	10	60
*Not fluorescent.			
		36-hr. culture	
1	860,000	283,000	35,230,000†
3	333,000	18,500	6,600,000
5	15,500	1,430	980,000
7	2,260	400	222,500
10	1,550	60	39,000
20	225	40	11,250
40	7	2	235
†Fluorescent.			

The conclusions drawn from this study are as follows:

In actively growing cultures, the variation in resistance with the age up to 72 hours in the absence of pigment is negligible. The cultures of the *Pseudomonas* organisms are more resistant to ultra-violet than those of *A. aërogenes* and *E. coli.* Because the pigment is diminished by dilution, the *Pseudomonas* organisms lose resistance to ultra-violet. In the undiluted cultures of *Pseudomonas*, the resistance increases with the amount of pigment present. The resistance of cultures of *Pseudomonas* which have lost the power to produce pigment is less than that of cultures which retain the power to produce pigment.

9783

Effect of Yeast and Nicotinic Acid on Porphyrinuria.*

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It has been shown by Beckh, Ellinger and Spies[1] that pellagrins have increased amounts of coproporphyrin I or III in their urine which disappear following the successful treatment of the pellagra by means of large amounts of yeast or liver extract. Since nicotinic acid had been shown to be effective in curing the mucous membrane lesions of pellagra,[2] it seemed desirable to determine whether or not it would decrease the porphyrinuria of pellagra and other diseases.

Specimens of urine were obtained from 4 groups of people (Table I). Group I included a patient with gonorrheal arthritis who had sulphanilamide treatment, a patient with diabetic coma, one with catarrhal jaundice, and one with alcoholic cirrhosis and ascites. Group II included 2 patients, one with barbital intoxication, and one with hypertensive heart disease, congestive heart failure, and an enlarged, tender liver. Group III included 2 cases of pellagra, one very severe, and the other mild. In Group IV were 6 normal persons who ate large quantities of a well-balanced diet.

The studies were conducted in such a manner that the source of the specimen of urine for analysis was unknown to the person examining it. Whenever positive tests were obtained, repeated observations were performed, usually by more than one examiner. After increased porphyrinuria had been demonstrated repeatedly, yeast or nicotinic acid was administered and the urine collected and examined as before. As indicated in the table, nicotinic acid, in doses of 500 mg., was administered to the persons in Group I and Group III, and yeast, in 50 gm. amounts, was given to the 2 patients in Group II. After the yeast or nicotinic acid had been given, and the test for porphyrinuria had become negative, the yeast or nicotinic acid was discontinued without any other changes. Subsequent urine

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* These studies were made possible by a grant from the David May Fund of the College of Medicine, University of Cincinnati.

[1] Beckh, Walter, Ellinger, Philipp, and Spies, Tom D., *Quar. J. Med.*, New Series, 1937, **6**, 305.

[2] Spies, Tom D., Cooper, Clark, and Blankenhorn, M. A., *The Use of Nicotinic Acid in the Treatment of Human Pellagra*, read before the Central Society for Clinical Research in Chicago, Illinois, November 5, 1937.

TABLE I.
Summary of Data.

Case	Clinical Diagnosis	Days 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
	Group I																									
1 C.M.	Gonococcal arthritis	+	+	+	+↑	—	—	—	—	+	+↑	—	—	—	—	+	+↑	—								
2 C.S.	Diabetic coma	+	+	+	+↑	—	—	—	+	+↑	—	—														
3 R.M.	Catarrhal jaundice	+↑	—	—	+	+↑	—	—	—																	
4 C.R.	Alcoholic cirrhosis with ascites	+		+	+	+		+	+		+	+	+	+	+	+	+↑	—	—	—	—	—	—	+↑	—	—
	Group II																									
5 F.B.	Barbital intoxication	+↑	—	—								—	—	—												
6 M.B.	Hypertensive heart disease with congestive failure	+	↑	+	—	—↑	↑	—↑	—↑	—↑	—↑	—↑	—↑	—												
	Group III																									
7 G.S.	Pellagra	+↑	+↑	↑	↑	+↑	↑	↑	↑	+↑	↑	—↑	—↑													
8 P.L.	Mild pellagra	+↑	—																							

+ = Positive test for porphyrinuria.
— = Negative test for porphyrinuria.
↑ = 500 mg. nicotinic acid in Groups I and III.
↑ = 50 gm. Brewers' Yeast (Harris) in Group II.

samples were examined. The method used for testing the urine was the same as that described by Beckh, Ellinger and Spies[1] for the estimation of ether-soluble porphyrin in small amounts in the urine.

As shown in the table, all persons with increased porphyrinuria gave a negative test for porphyrinuria following the administration of either nicotinic acid or yeast. The test for porphyrinuria became negative in 7 of the patients within 24 hours after the administration of either nicotinic acid or yeast. In the case of severe pellagra the porphyrinuria steadily decreased from the time the nicotinic acid was administered, but the test remained positive for several days. In the case of mild pellagra the test became negative over night following the administration of nicotinic acid. The urine specimens from the 6 persons who ate large amounts of a well-balanced diet always gave negative tests for porphyrinuria.

The present study confirms the observations of Beckh, Ellinger and Spies[1] that pellagrins have increased porphyrinuria. In addition these studies show that the administration of yeast was followed by the disappearance of porphyrin in the urine of a case of barbital intoxication and of another case with cardiac decompensation. Also, it has been shown in these studies that nicotinic acid likewise remits porphyrinuria in pellagra and in certain other diseases. Since there is a similarity between the clinical symptoms of pellagra and those of acute porphyrinuria, the presence of an excessive amount of porphyrin in the body of pellagrins might explain some of the clinical symptoms. If the increased porphyrin of the urine of pellagrins proves to be coproporphyrin I, we must consider the possibility of a complete new synthesis of porphyrin as suggested by Fischer and Duesberg.[3] If, on the other hand, it is coproporphyrin III, we may assume a connection with an abnormal hemoglobin metabolism. In the functionally impaired liver bilirubin eventually may be broken down into coproporphyrin III. It seems to the authors that the latter explanation is the most probable one, and it is consistent with the observation[1] that patients dying from pellagra frequently show anatomical changes in the liver. We must admit, however, that there are many discrepancies between anatomical and functional liver changes.

Summary and Conclusions. 1. These studies confirm the previous observations of Beckh, Ellinger and Spies[1] that porphyrinuria is a frequent finding in pellagra and disappears following the administration of yeast. 2. The present study shows that nicotinic acid, a substance which has been shown to be curative for the mucous membrane lesions of pellagra, likewise produces a decrease of porphyrinuria in pellagrins. 3. In addition, the present study shows that the porphyrinuria associated with a number of diseases other than pellagra likewise is promptly decreased following the administration of either yeast or nicotinic acid.

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[3] Fischer, H., and Duesberg, R., *Arch. f. exp. Path. u. Pharm.*, Berlin, 1932, **166**, 95.

9784 P

Intravenous Benzedrine Sulfate as an Antagonist to Intravenous Soluble Amytal.

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During our previous investigations of the stimulating action of benzedrine sulfate on the central nervous system[1, 2, 3] the possibility suggested itself of employing this drug to counteract the narcosis produced by barbiturates. Alles[4] observed that benzedrine intravenously had considerable effect in waking animals from barbital anesthesia. In man, Myerson, *et al.*,[5] reported that benzedrine sulfate subcutaneously did not affect the depth, although it definitely shortened the duration of soluble amytal narcosis; and stated that hypertension produced by benzedrine sulfate subcutaneously could be reduced by soluble amytal intravenously, and also that hypotension produced by soluble amytal intravenously could be elevated by benzedrine sulfate subcutaneously. In rats, rabbits and guinea pigs unprotected by barbiturates the minimum lethal dose subcutaneously or intravenously has been reported by all observers[4, 6, 7] to be at least 25 mg. per kg. body weight. On the basis of these determinations it was felt that the dosage herein employed was entirely safe.

In the present study we decided to employ both soluble amytal and

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[1] Davidoff, E., *Psychiat. Quart.*, 1936, **10**, 652.

[2] Davidoff, E., and Reifenstein, E. C., Jr., *J. A. M. A.*, May 22, 1937, **108**, 1770.

[3] Davidoff, E., and Reifenstein, E. C., Jr., *J. Lab. and Clin. Med.*, to be published.

[4] Alles, G. A., *J. Pharm. and Exp. Therap.*, 1933, **47**, 339.

[5] Myerson, A., Loman, J., and Dameshek, W., *Am. J. M. Sci.*, 1936, **192**, 560.

[6] Hartung, W. H., and Munch, J. C., *J. Am. Chem. Soc.*, 1931, **53**, 1875.

[7] Ehrich, W. E., and Krumbhaar, F. B., *Ann. Int. Med.*, 1937, **10**, 1874.

benzedrine sulfate intravenously. Ten patients without apparent physical disease were selected. Each was subjected to a control period as follows: the usual blood pressure and pulse levels were determined; then soluble amytal, 0.5 gm. (7½ grains), was given intravenously; the narcosis thus induced was allowed to terminate spontaneously; the duration of the narcosis was noted; and the blood pressure and pulse levels were again determined at the moment of awakening. Several days later the same patients were subjected to the following test period: the initial blood pressure and pulse levels were determined; soluble amytal, 0.5 gm. (7½ grains) was given intravenously; after a period of narcosis varying from 15 to 45 minutes the clinical state of the narcosis and the blood pressure variation were observed; thereafter benzedrine sulfate 10 mg. intravenously was repeated at intervals of approximately 15 minutes until the state of narcosis disappeared, and until the systolic blood pressure exceeded 150 mm. of mercury; the length of time and the amount of benzedrine sulfate necessary to overcome the soluble amytal narcosis was recorded; finally, the blood pressure and pulse variations were observed until the original levels obtained.

The results are summarized in Tables I and II. Both the depth and the duration of the soluble amytal narcosis were affected, as in every case the narcosis was completely counteracted by 20 or 30 mg. of benzedrine sulfate intravenously, and in 5 instances by only 10 mg. Every patient awakened following the initial injection of 10 mg. of benzedrine sulfate, but some remained drowsy until the third injection was completed. Almost all the patients became talkative, some were euphoric, others began to weep bitterly, and a few were restless, tremulous and complained of headache. Every patient was up and about within an hour of the first benzedrine injection.

TABLE I.
Effect of Intravenous Benzedrine Sulfate on Soluble Amytal Narcosis: Duration of Narcosis and of Blood Pressure Variations.

	Aver. Duration, Min.	Range of Duration, Min.
Control Period	257	155-366
Prior to Benzedrine Sulfate Injection	35	17-52
After Benzedrine Sulfate Injection (as indicated by drowsiness)		
(a) 6 Cases (10 mg.)	4	2-5
(b) 4 Cases (20-30 mg.)	37	28-47
Period of Blood Pressure Rise (Initial Benzedrine Sulfate injection to maximum level)	66	5-125
Period of Blood Pressure Fall (Final Benzedrine Sulfate injection to original level)	215	85-417

TABLE II.

Effect of Intravenous Benzedrine Sulfate on Soluble Amytal Narcosis: Blood Pressure and Pulse Alterations

	Prior to Soluble Amytal Narcosis		Prior to Benzedrine Sulfate Injection		After Benzedrine Sulfate Injections					
					10 mg.		20 mg.		30 mg.	
Case No.	B.P.†	P.‡	B.P.	P.	B.P.	P.	B.P.	P.	B.P.	P.
1. (F.A.)	150/70	78	110/60	80	200/100	60	198/98	66	—	—
2. (W.V.)§	100/62	86	108/68	68	112/66	70	142/88	88	152/88	74
3. (F.J.)	148/80	100	112/74	96	136/90	80	148/84	72	160/80	100
4. (R.R.)	100/68	86	96/60	76	120/70	64	150/80	60	164/82	54
5. (M.M.)	122/64	80	98/60	80	132/88	70	146/90	58	186/94	54
6. (S.S.)	124/70	70	102/60	64	116/80	64	166/92	80	—	—
7. (J.P.)	142/76	90	115/85	96	154/100	98	180/100	60	218/106	68
8. (H.R.)§	140/82	96	105/70	84	145/105	76	150/100	68	174/100	60
9. (H.A.)	108/72	68	95/62	100	110/80	78	130/90	60	152/94	58
10. (L.P.)	120/75	132	50/0	*	140/90	88	140/100	90	150/90	100

*Patient in collapse, pulse too rapid to be counted; Benzedrine Sulfate 10 mg. injected intravenously at once, with immediate response of patient.

†Blood Pressure. ‡Pulse. §After 40 mg.: Case 2—162/86, 68; Case 8—182/102, 70.

The variations of the blood pressure and the pulse were striking. There was usually a fall of the systolic and the diastolic blood pressure levels and of the pulse following the injection of soluble amytal. Attention is directed to the response in Case 10. In every case 10 mg. of benzedrine sulfate restored the blood pressure to approximately the pre-narcosis level, although the pulse tended to remain slow or become slower. In Case I marked hypertension was produced with extreme rapidity. In other cases, 20 to 30 mg. of benzedrine sulfate raised the systolic level 50 to 100 points with a 20 to 40 increase in the diastolic level. Extremely high levels were maintained for only a short time, after which a gradual fall occurred to pre-narcosis levels which were reached in every case under 7 hours. In most instances the pulse became slower as the blood pressure rose. It must be pointed out that the blood pressure and the pulse variations in the test period are a response to both benzedrine sulfate and soluble amytal.

Although benzedrine sulfate seemed to exert an antagonistic action to soluble amytal, the narcosis produced by 0.5 gm. (7½ grains) intravenously in some of the patients studied was not as deep as we desired. The needle-prick accompanying the benzedrine sulfate injection produced a momentary response in some patients and must therefore be considered a source of error.

Benzedrine sulfate merits further investigation as a therapeutic aid in barbital intoxications.

We wish to express appreciation to the Smith, Kline and French Laboratories for supplying benzedrine sulfate, and the Eli Lilly Research Laboratories for soluble amytal used in this study.

9785

Anaphylactic Shock Prevented by Ketenizing Serum.*

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Our investigations[1] concerning the action of ketene ($CH_2 = CO$) on the endotoxin-producing bacteria (*B. dysenteriæ* Shiga) proved that ketene detoxified the bacteria without altering the antigenic properties of the organisms. Subsequent research (unpublished) on such toxins as diphtheria, tetanus, and ricin, has established the fact that ketene is an effective detoxifying agent. The mechanism of the detoxification involves the chemical reaction between ketene and the primary amino groups of the toxin, with the formation of an acetylated toxin.

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* This research was aided in part by a grant from the Craig Yeiser Fund, College of Medicine, University of Cincinnati.

[1] Tamura, J. T., and Boyd, M. J., *Science*, 1936, **83**, 61.

Recently, Goldie and Sandoz[2] have studied the influence of ketene on the antigenic and the anaphylactogenic (shock producing) properties of diphtheria antitoxin. They conclude that ketene-treated diphtheria antitoxin is modified to some extent by a change in its chemical structure. The anaphylactogenic power of the antiserum undergoes nearly complete attenuation while the major part of its antitoxic properties remains unaltered.

Anti-Brucella horse serum and young healthy guinea pigs were used in our investigations. All the animals were sensitized to antiserum diluted 1:10, each one receiving 0.5 cc. subcutaneously. To each 10 cc. lot of the original antiserum to be ketenized, 0.5 gm. of $NaHCO_3$ was added to buffer the acetic acid which is formed during the process. A few drops of caprylic alcohol were introduced to prevent foaming. Ketene gas was passed through the antiserum for various lengths of time at the rate of about one bubble per second (diam. of inlet tube, 4 mm.). At the end of the ketenizing process the pH of the antiserum was determined by the glass electrode method and it was found to be between 6.7 and 6.3 for antiserum treated 35 and 40 minutes respectively. Sufficient $NaHCO_3$ was added immediately, usually 0.1 gm. to adjust the pH to 7.0. The control antiserum was made by adding the same amount of $NaHCO_3$ to the original antiserum and adjusting the pH to 7.0 by addition of acetic acid. Both the control and the ketenized antisera were treated with the bicarbonate for approximately the same length of time. Since a slight precipitation of protein occurred during the ketenizing process the protein content of each lot of precipitate-free antiserum was determined by kjeldahl.

Two weeks after sensitizing the guinea pigs to the original antiserum, a shocking dose of ketenized antiserum was injected intracardially into one group of the animals and the control antiserum given likewise to another group. The results of this experiment are given in Table I.

Eleven animals showed very mild signs of shock or no anaphylac-

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[2] Goldie, H., and Sandoz, G., *Comptes Rendus des Séances de la Société de Biologie*, 1937, **126**, 291.

TABLE I.
Results in Guinea Pigs Previously Sensitized to Anti-Brucella Horse Serum and Later Injected Intracardially with Original and Ketenized Antiserums.

Serum	No. of Guinea Pigs	Amount of shocking dose injected		Results
		cc.	mg. protein	
Control	1	.5	38.5	Shocked. Died in 3 min.
	2	.5	38.5	" " " 2.5 "
	3	.5	38.5	" " " 4 "
	4	.4	26.6	" " " 2 "
	5	.3	19.9	" " " 3.5 "
	6	.2	13.9	" " " 2 "
	7	.2	13.9	" " " 3.5 "
Ketenized 15 min.	1	.5	38.5	" " " 3 "
" 30 "	1	.5	38.5	" " " 5.5 "
	2	.5	38.5	" " " 12.5 "
	3	.5	38.5	Shock delayed. Recovered
	4	.5	38.5	Syndrome in lesser degree. Recovered.
" 35 "	1	.4	28.0	Very mild dyspnea for few minutes. Recovered.
	2	.5	32.5	Very mild dyspnea for few minutes. Recovered.
	3	.5	32.5	No anaphylactic syndrome
	4	.5	32.5	" " "
	5	.5	35.0	" " "
	6	.6	42.0	" " "
" 40 "	1	.5	33.7	Mild dyspnea—scratching nose
	2	.5	33.7	No anaphylactic syndrome
	3	.5	33.7	" " "
	4	.75	50.2	" " "
	5	1.0	67.0	Mild dyspnea—scratching nose

tic syndrome when injected with antiserum that had been treated with ketene for 35 minutes or more, containing from 28 mg. to 67 mg. of protein per dose. Seven control animals died within 2 to 4 minutes with the usual severe shock when injected with the control serum containing 13.9 mg. or more of protein per dose.

TABLE II.
Agglutinating Power of the Antiserums, Before and After Ketenization.

Serum	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Original	4+	4+	4+	4+	4+	4+	4+	4+	—
Ketenized 15 min.	4+	4+	4+	4+	4+	4+	4+	4+	—
Ketenized 30 min.	4+	4+	4+	4+	4+	4+	4+	3+	—
Ketenized 35 min.	4+	4+	4+	4+	4+	4+	4+	2+	—
Ketenized 40 min.	4+	4+	4+	4+	4+	4+	4+	2+	—

The antibody content of the serums used in this investigation was determined by titration of the agglutinin titer against *Brucella abortus* suspensions.

The results of the test given in Table II show that the major part of the agglutinins were still intact after ketenization.

Conclusion. The treatment of anti-Brucella horse serum with ketene for 35 minutes or more prevents anaphylactic shock in animals sensitized to the original antiserum. Such a serum still retains the major part of its agglutinating antibody.

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Pathogenesis of Hemorrhagic-Necrotic Skin Lesions in Intradermal Infection of Rabbits with Pneumococci.*

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The hemorrhagic necrotic changes occurring in certain endermal infections of rabbits closely resemble those described by Shwartzman. This induced us to examine various species of pathogenic microörganisms as to their capacity to produce the lesion in question. Previously reported experiments[1-4] revealed 3 different types of reactions represented by the following bacteria:

(1) *H. influenzæ,* when injected into the skin over the abdomen produces a localized swelling and infiltration. There is no trace of hemorrhagic necrosis to be observed. When, 24 hours after the intracutaneous injection of *H. influenzæ,* a suspension of *H. influenzæ* is given intravenously, the site of the dermal infection may be transformed into a bluish hemorrhagic lesion within a few hours. Not only living and heat-killed influenza bacilli but also agar-washing filtrates of *B. typhosus, B. coli,* or meningococcus may induce the same change. On the other hand, those filtrates may be activated by the intravenous injection of *H. influenzæ.* (2) Some

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[1] Witebsky, E., and Salm, H., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 351.

[2] Witebsky, E., and Salm, H., *J. Exp. Med.*, 1937, **65**, 43.

[3] Witebsky, E., Neter, E., and Salm, H., Second International Congress for Microbiology, London, Report of Proceedings, 1936, 414.

[4] Witebsky, E., and Neter, E., *Arch. Path.*, 1937, **24**, 271.

strains of Friedländer bacilli spontaneously may induce the formation of hemorrhagic necrotic skin lesions in rabbits. When a heat-killed suspension of such a strain is injected intracutaneously into a rabbit, the site of the infection may be transformed into a hemorrhagic necrotic lesion by means of a subsequent intravenous injection of a suspension of living or heat-killed Friedländer bacilli. (3) Pneumococci, finally, display a very interesting picture: When injected intracutaneously certain rabbit-virulent strains produce a severe dermal lesion followed by hemorrhagic necrosis and accompanied by bacteremia as described by Goodner. The following experiments are concerned with the experimental conditions which would bring about the hemorrhagic change of the lesion in the dermal pneumococcal infection of rabbits.

For this purpose rabbits were inoculated intracutaneously with a highly virulent strain of Type I pneumococcus (obtained through the courtesy of Dr. Paul R. Cannon). Twenty-four hours later, the rabbits showed a severe lesion characterized by swelling and œdema, followed by hemorrhagic necrosis and death of the animals after 2 to 4 days. The influence of an intravenous injection of a potent *B. typhosus* agar-washing filtrate prepared according to Shwartzman is shown by the following experiment:

Six rabbits (Nos. 1-6) weighing about 4-5 pounds, were shaved over the abdomen; then 0.2 cc. of a 1:50 diluted 18-hours meat-infusion broth culture of pneumococcus Type I was injected intracutaneously. Eighteen hours later all the rabbits exhibited severe swelling and œdema at the site of the dermal infection without any trace of hemorrhagic changes. At this stage 3 rabbits (Nos. 1, 2, and 3) were injected intravenously with 2 cc. of a 1:20 diluted *B. typhosus* agar-washing filtrate. Rabbits Nos. 4, 5, and 6 were used as controls.

The results of this experiment (Table I) show that the injection of Shwartzman filtrate may bring about a severe hemorrhagic necrotic lesion at the site of the pneumococcal infection, the intensity and development of which exceeds that of the untreated rabbits. The 3 rabbits injected with *B. typhosus* filtrate died in 12-24 hours previous to the controls. Similar observations were made on several occasions.

In contrast, heat-killed pneumococci of the same strain when injected endermally, apparently did not prepare the skin for a subsequent transformation into a hemorrhagic lesion when living pneumococci or Shwartzman filtrate respectively were injected intravenously 24 hours later. In this respect pneumococci differ

TABLE I.
Production of Hemorrhagic Necrosis in Skin Areas of Rabbits Infected with Pneumococcus Type I.

Hours after reinjected with Shwartzman filtrate	A			B Controls		
Rabbit No.	1	2	3	4	5	6
1 hr.	+	++++	+	—	—	—
4 hr.	++++	++++	++++	+	—	—

— = no hemorrhagic necrosis.
+ to ++++ = various degrees of hemorrhagic necrosis.

markedly from *H. influenzæ*. It was impossible thus far to use successfully living or heat-killed pneumococci for the second—intravenous—injection in order to produce hemorrhagic necrotic lesions in the skin previously prepared by the intracutaneous injection of living rabbit-virulent pneumococci. On the other hand, rabbits previously injected intradermally with pneumococci developed hemorrhagic reactions following the intravenous injection of bacterial filtrates, 0.2% agar and occasionally of heavy suspensions of killed pneumococci as reported by Wadsworth and Sickles.[5] Goodner and Horsfall[6] recently produced a purpuric reaction in a skin-area infected with Type I pneumococcus by means of pneumococcal autolysate prepared according to the technic described by Julianelle and Reimann.[7]

There are some strains of pneumococci that produce a certain degree of swelling but fail to cause a hemorrhagic necrotic lesion in the skin of the rabbit and do not kill rabbits. The influence of bacterial agar-washing filtrates on such a lesion is shown in the following experiment:

Seven rabbits weighing about 4-5 pounds were shaved over the abdomen and injected with 0.2 cc. of a Type 3 pneumococcal suspension. Eighteen hours later the rabbits exhibited definite redness and swelling at the site of the infection without any hemorrhages or necrosis. Four of the rabbits (Nos. 1-4) were then injected intravenously with 2 cc. of a 1:20 diluted Shwartzman agar-washing filtrate. Rabbits Nos. 5, 6, and 7 were used as controls.

The results of this experiment show that it may be possible to induce a hemorrhagic change at the site of a pneumococcal skin-lesion that fails to develop spontaneously into a hemorrhagic necrotic lesion.

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[5] Wadsworth, A., and Sickles, G. M., *J. Bact.*, 1933, **25**, 80.

[6] Goodner, K., and Horsfall, F., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 178.

[7] Julianelle, L. A., and Reimann, H. A., *J. Exp. Med.*, 1926, **43**, 87, 97; 1927, **45**, 609.

TABLE II.

Production of Hemorrhagic Necrosis in Skin Areas of Rabbits Infected with Pneumococcus Type III.

Hours after reinjected with Shwartzman filtrate	A				B Controls		
Rabbit No.	1	2	3	4	5	6	7
4 hr.	++++	—	++	—	—	—	—
18 hr.	++++	+	++++	—	—	—	—

— = no hemorrhagic necrosis.

+ to ++++ = various degrees of hemorrhagic necrosis.

One would be tempted to draw the conclusion from these experiments that the severe bacteremia thus far usually considered to be nothing more than a symptom in the pneumococcal infections of rabbits—and human beings—may be a contributing etiological factor in the development of the hemorrhagic necrotic change of the primary lesion. Indeed, substances present in bacterial agar-washing filtrates, prepared according to Shwartzman, are able to induce the changes in question. The above-mentioned investigations of Wadsworth and Sickles and of Goodner and Horsfall point in the same direction. Living as well as dead pneumococci, however, when injected intravenously proved to be ineffective in our experiments so far. Further experiments are necessary in order to elucidate this problem.